Cell-to-cell communication

Cell-to-cell communication: molecular mechanisms

Lecture 2

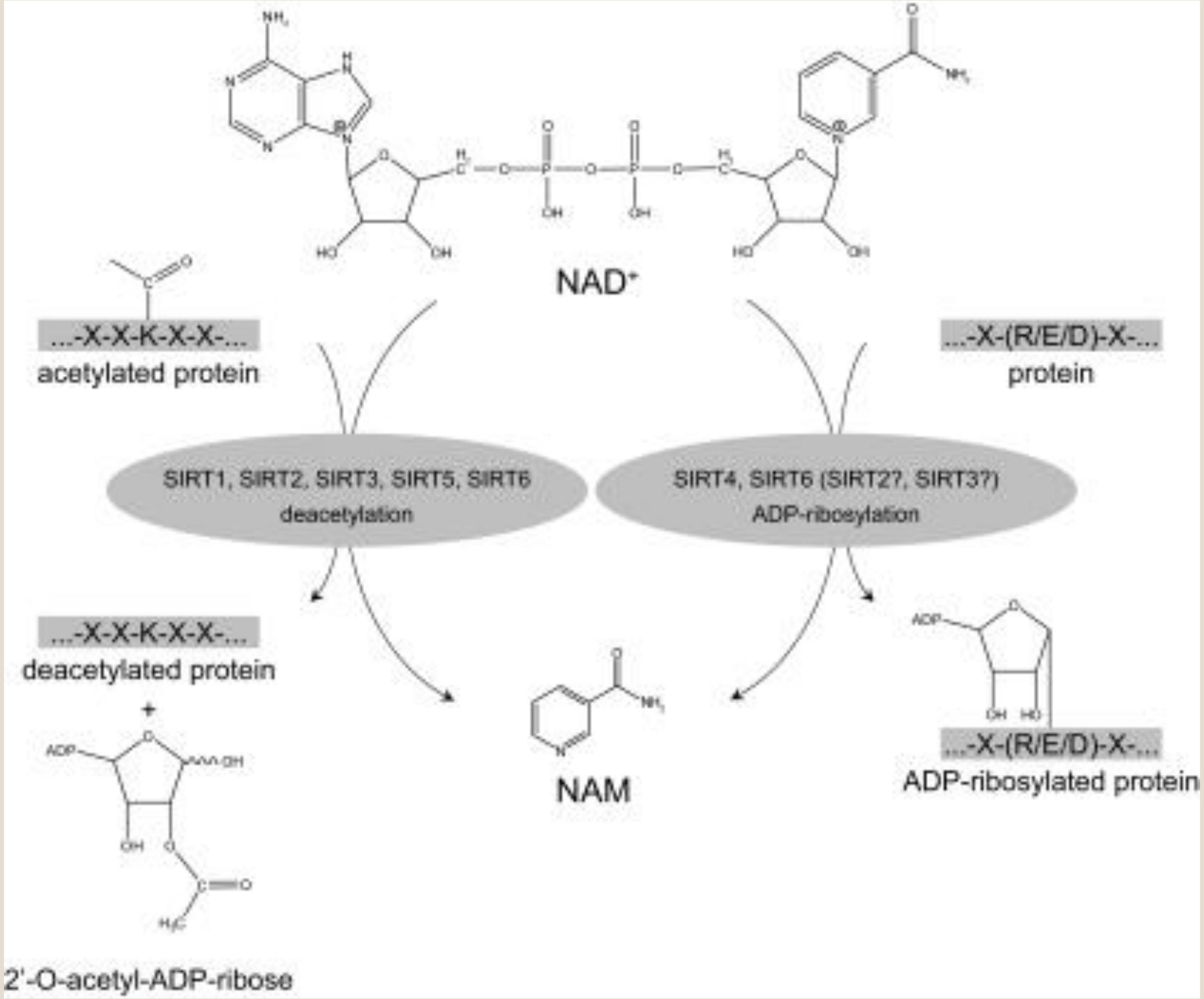
Parameters of signal transduction

1. **Signal molecules**: properties (hydrophobicity) → location of R (surface/ intracellular)
2. **Properties of receptors** (R): specificity to signal (type of signal → type of R); ability to transduce the signal (binding/ catalytic); adaptation of R to signal (desensitization; feedback regulation)
3. **Signaling pathway**: structure of adaptors; amplification of signal (2 strategies depending on surface/intracellular location of R); integration of signal pathways (cross-talk; coordination of response)
4. **Molecular targets and time necessary for response** (quick/prolonged depending on protein synthesis)
5. For multicellular organisms: **hierarchy of signal systems**; location-, dose- and time-dependent effects

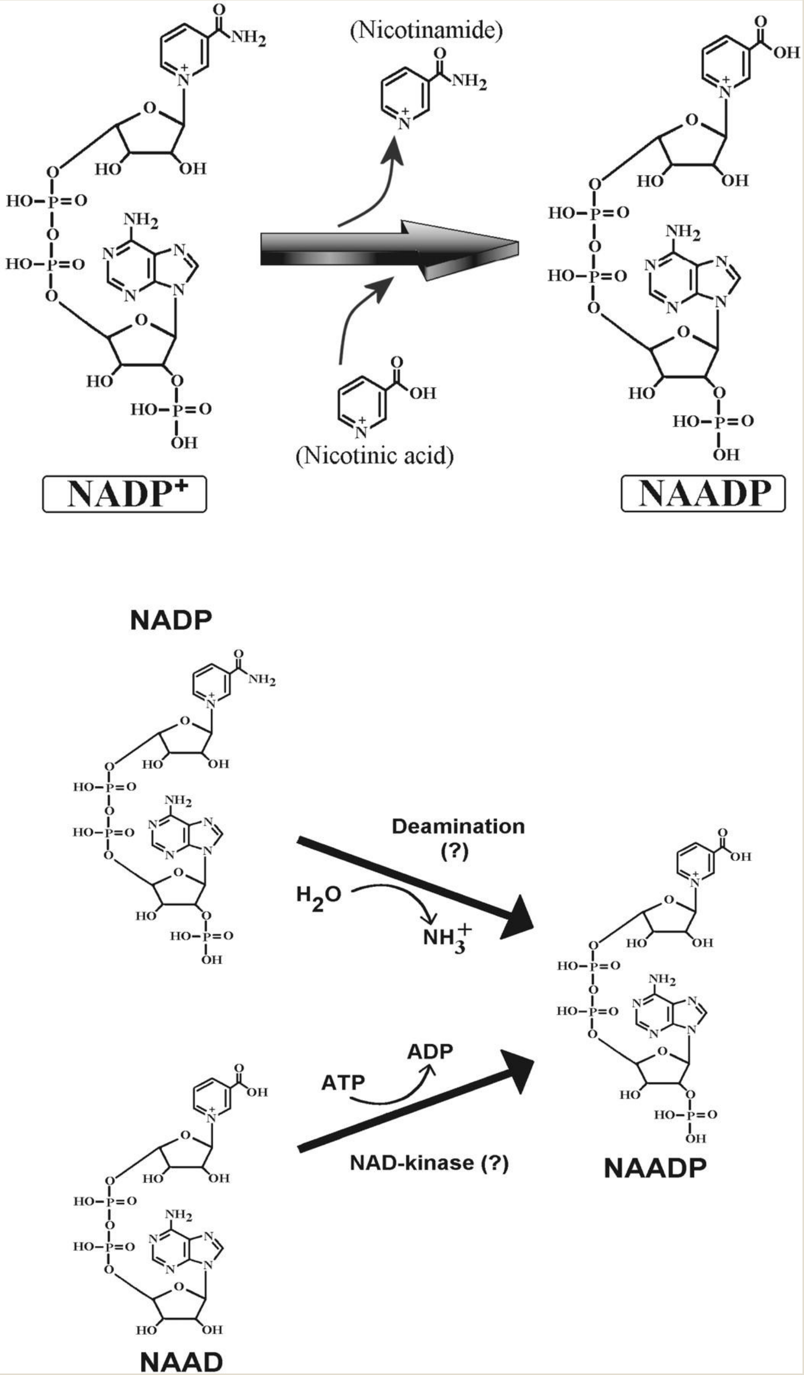
- NAD/NADH signaling
- ATP/ADP signaling
- Metabolic speciation

The Secret Life of NAD$^+$: An Old Metabolite Controlling New Metabolic Signaling Pathways

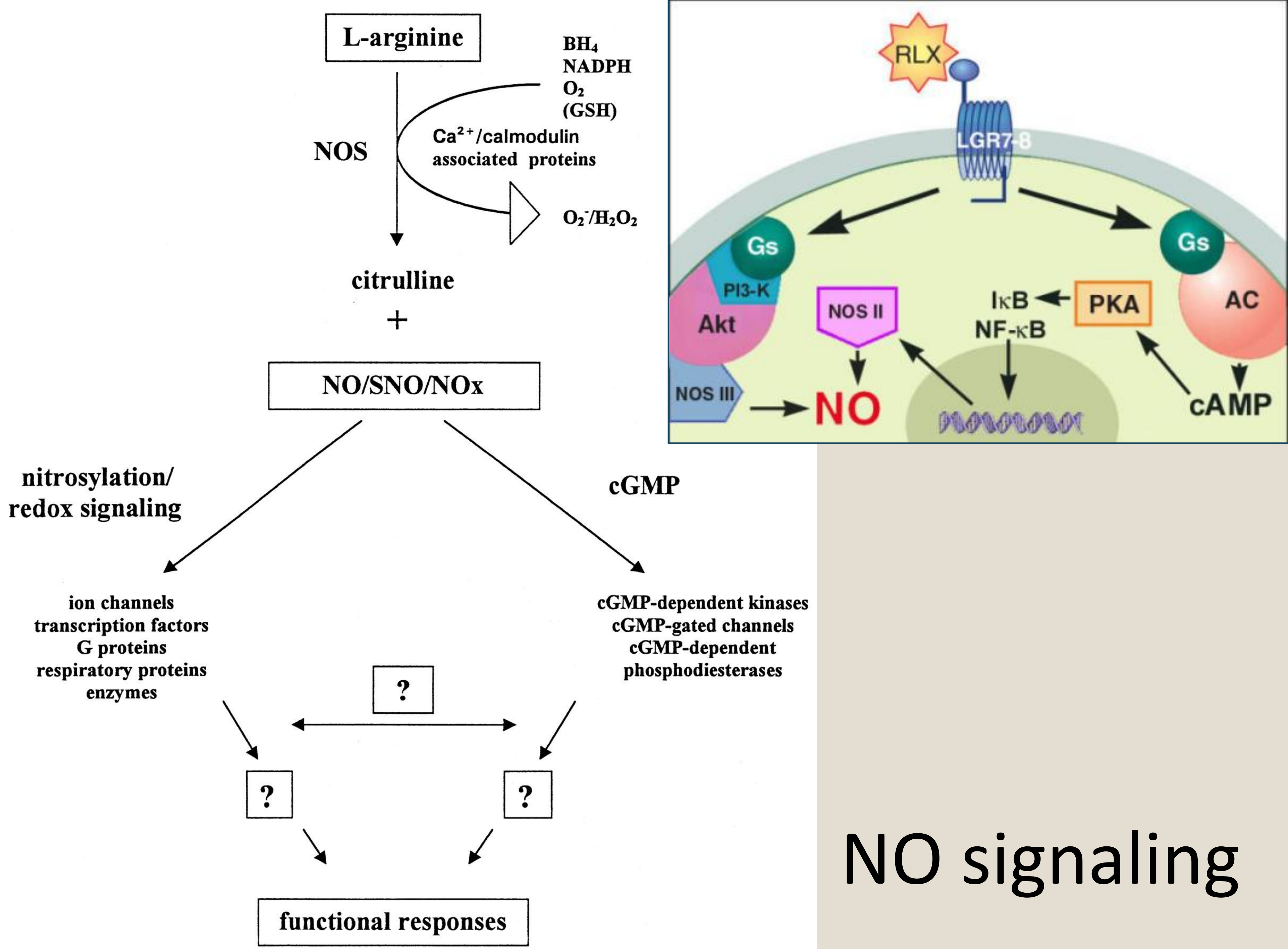
http://edrv.endojournals.org/content/31/2/194.long

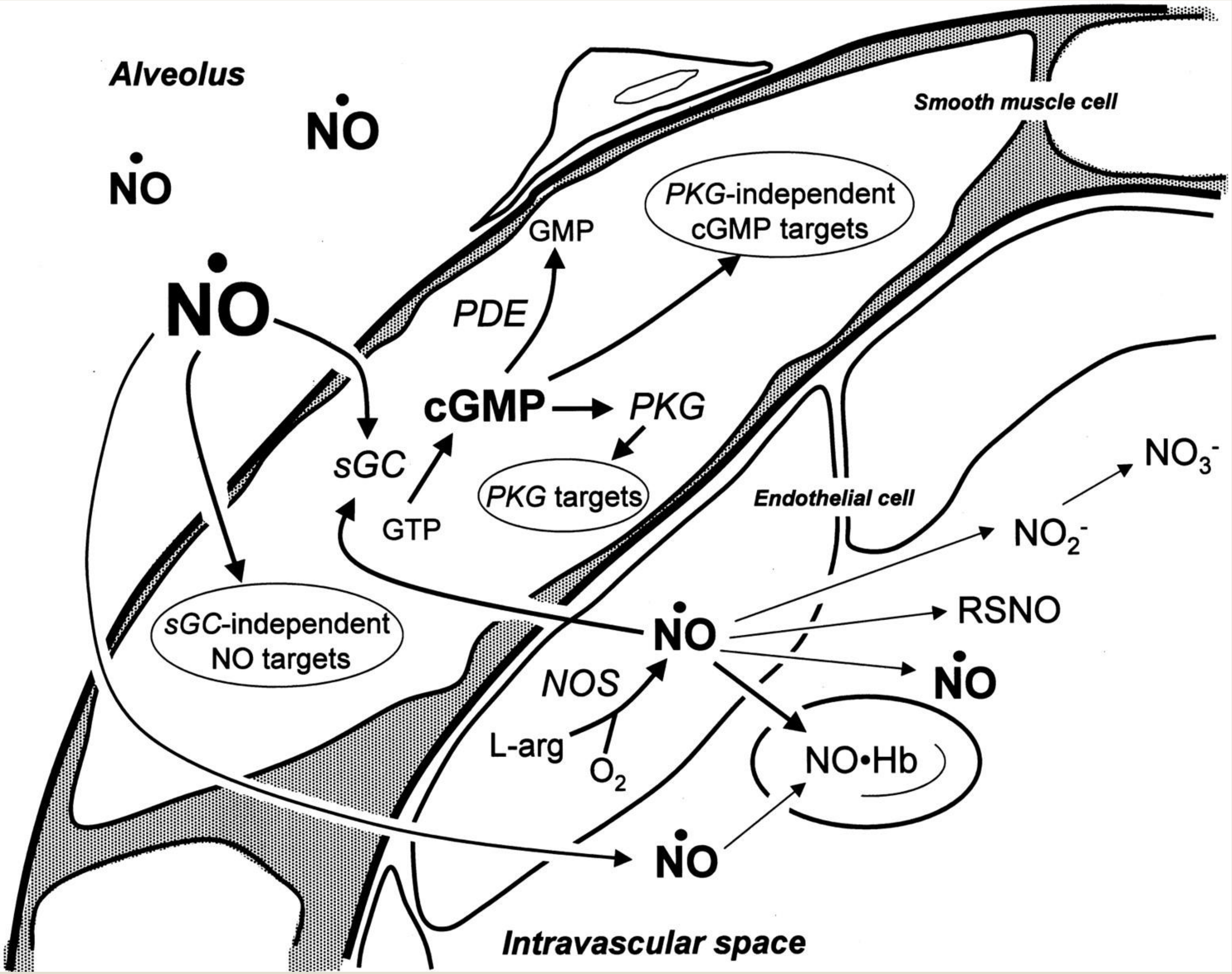


NAADP as a second messenger

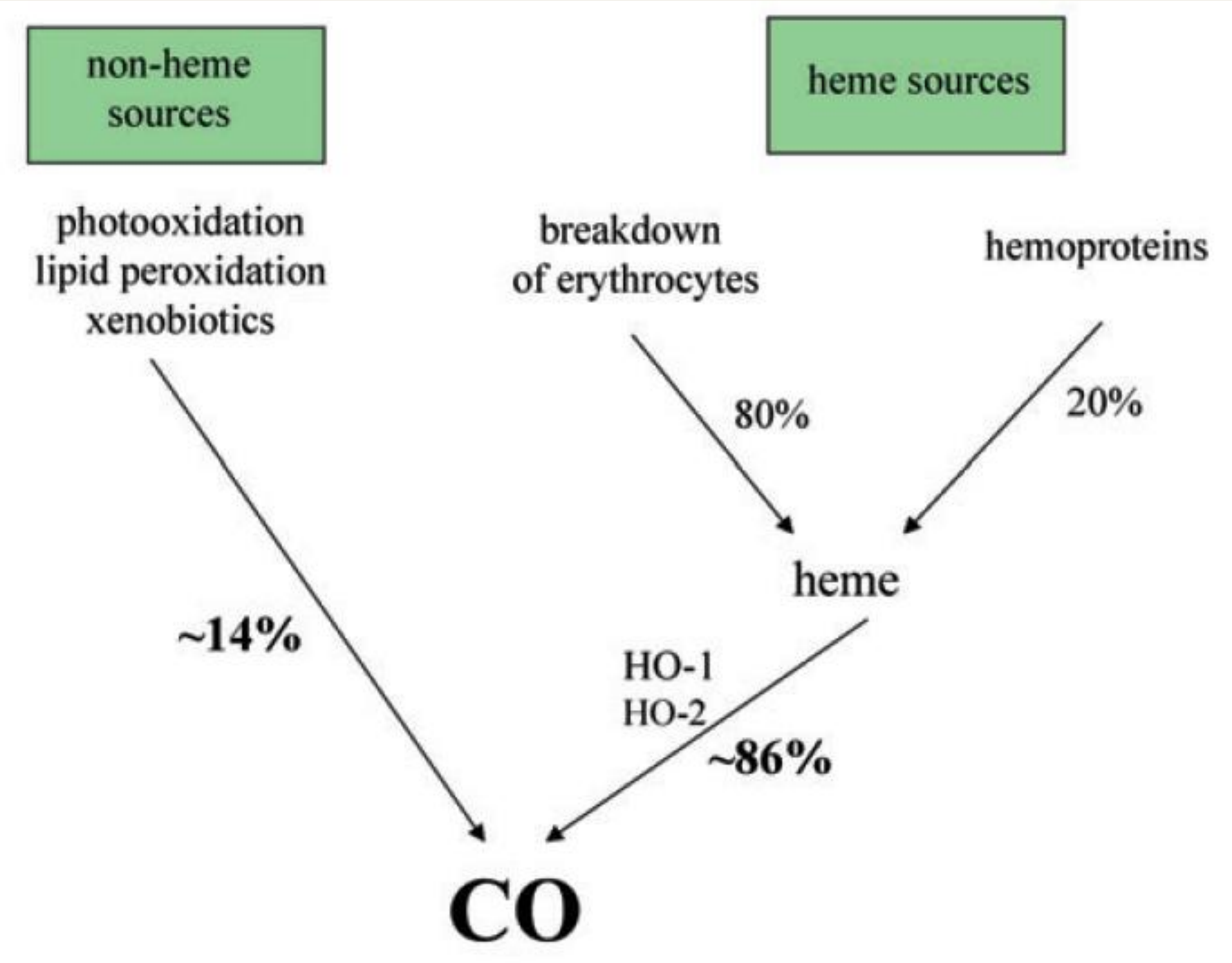


NO signaling




Alveolus
NO
NO
NO
Smooth muscle cell
PKG-independent cGMP targets
GMP
PDE
cGMP
PKG
sGC
PKG targets
GTP
Endothelial cell
NO₃⁻
NO₂⁻
sGC-independent NO targets
NO
RSNO
NO
NOS
L-arg
O₂
NO•Hb
NO
Intravascular space

Carbon monoxide -- a "new" gaseous modulator of gene expression



non-heme sources

heme sources

photooxidation
lipid peroxidation
xenobiotics

breakdown of erythrocytes

hemoproteins

80%

20%

heme

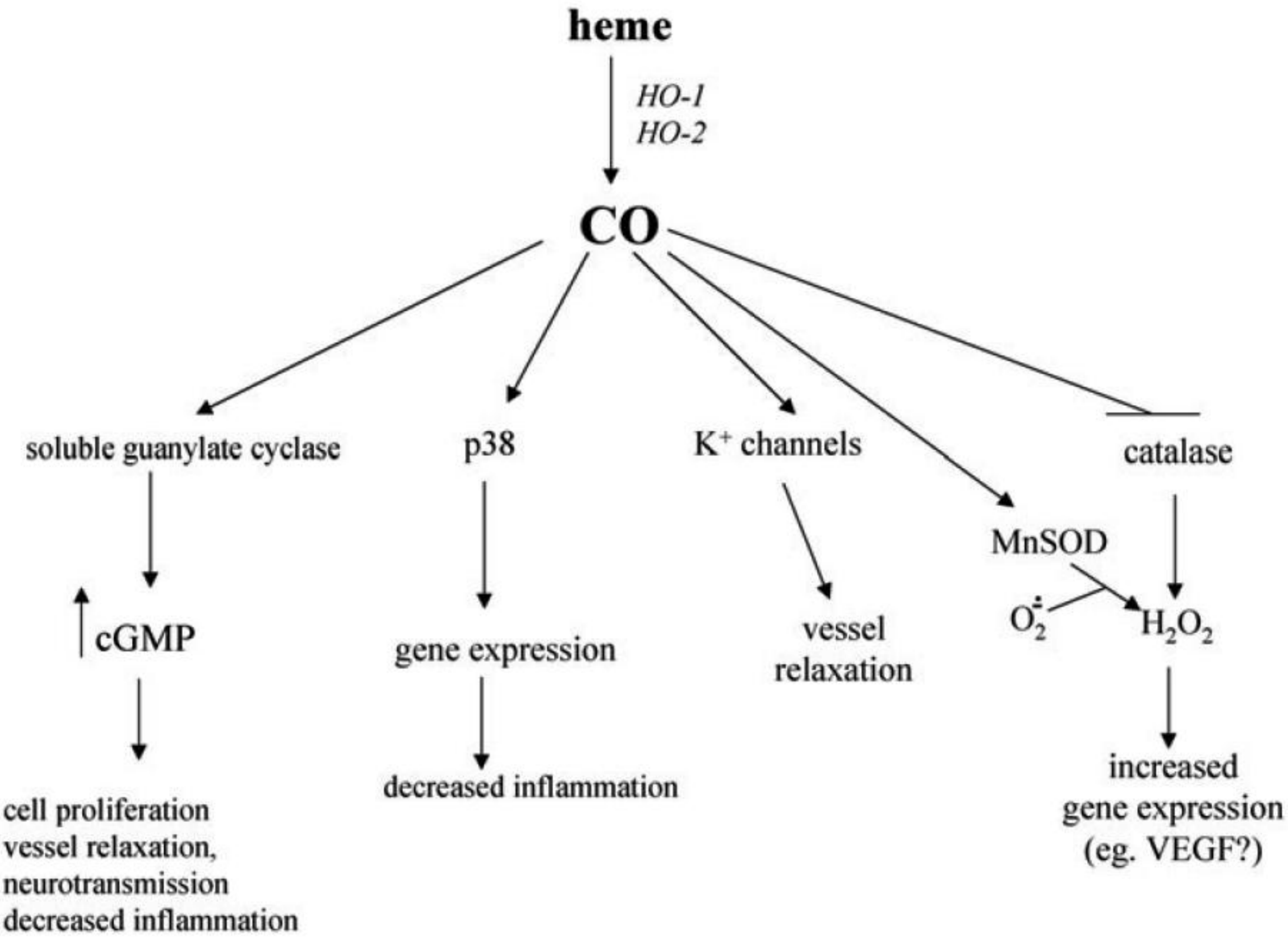
~14%

HO-1
HO-2

~86%

CO

The effect of CO on gene expression



heme

HO-1
HO-2

CO

soluble guanylate cyclase

↑ cGMP

cell proliferation
vessel relaxation,
neurotransmission
decreased inflammation

p38

gene expression

decreased inflammation

K^+ channels

vessel
relaxation

MnSOD

$O_2^{\cdot -}$

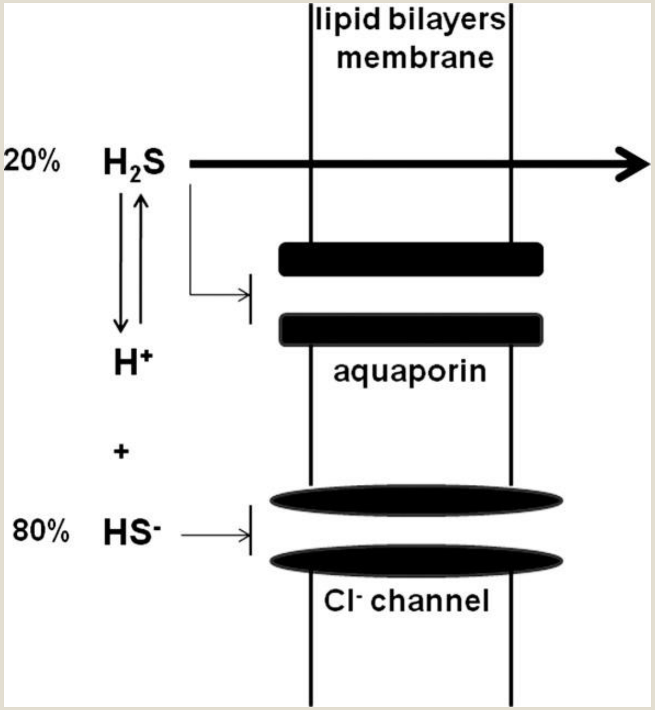
H_2O_2

catalase

increased
gene expression
(eg. VEGF?)

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3342561/

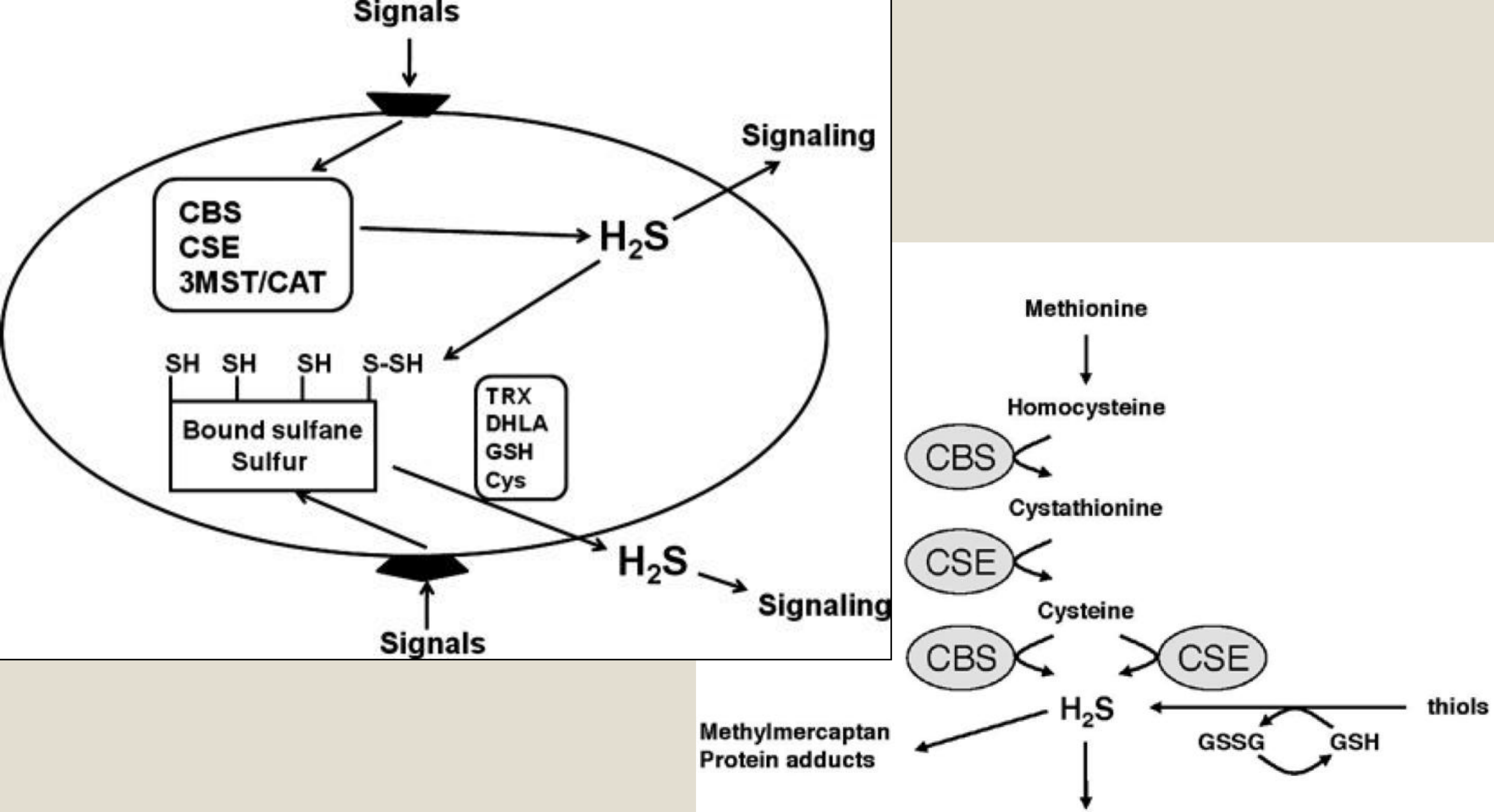
Hydrogen Sulfide Is a Signaling Molecule and a Cytoprotectant
Antioxid Redox Signal. 2012 July 1; 17(1): 45–57



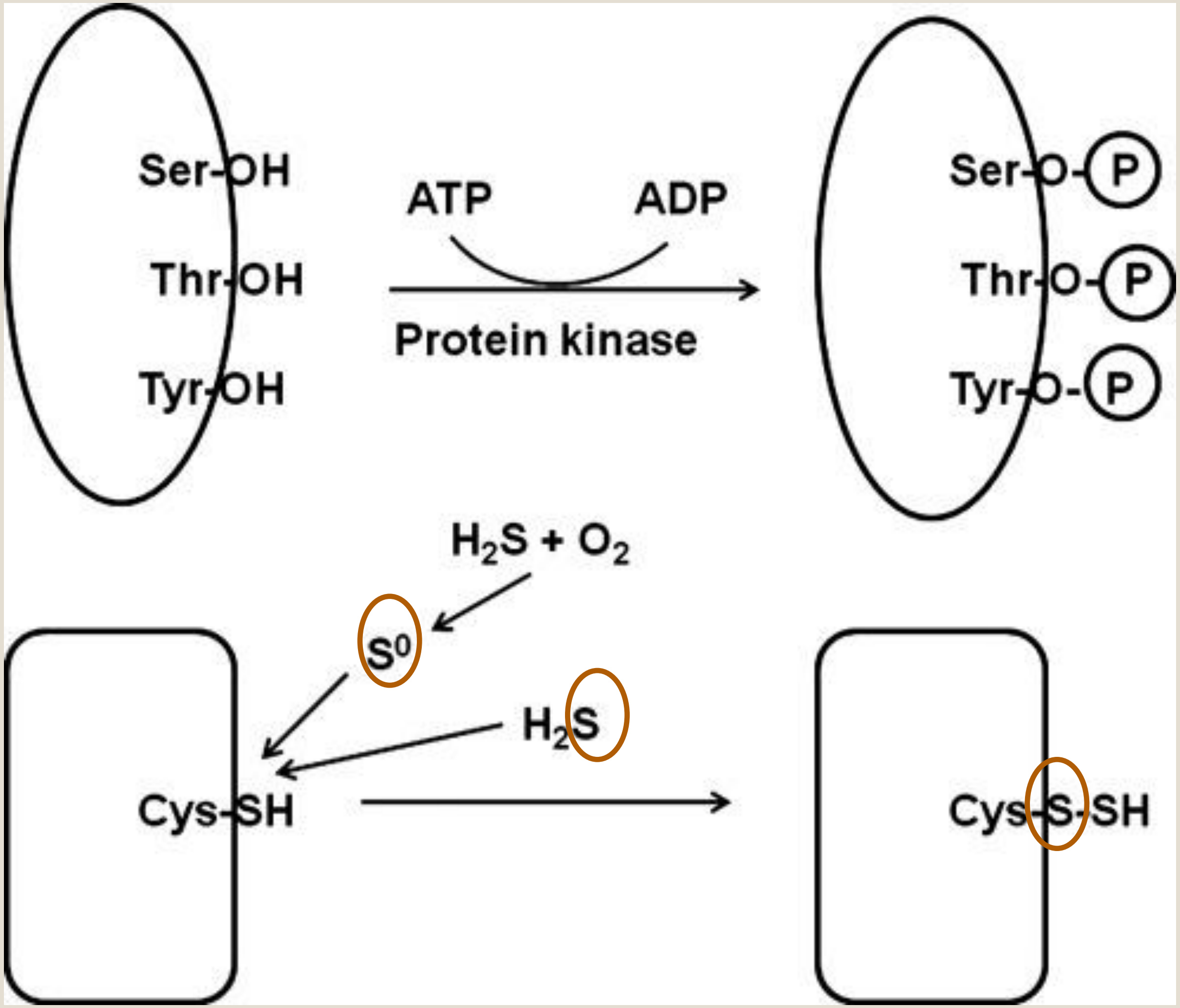
H2S hydrogen sulfide may function as a signaling molecule in processes such as neuromodulation in the brain and smooth muscle relaxation in the vascular system.

It also has a cytoprotective effect, since it can protect neurons and cardiac muscle from oxidative stress and ischemia-reperfusion injury, respectively.

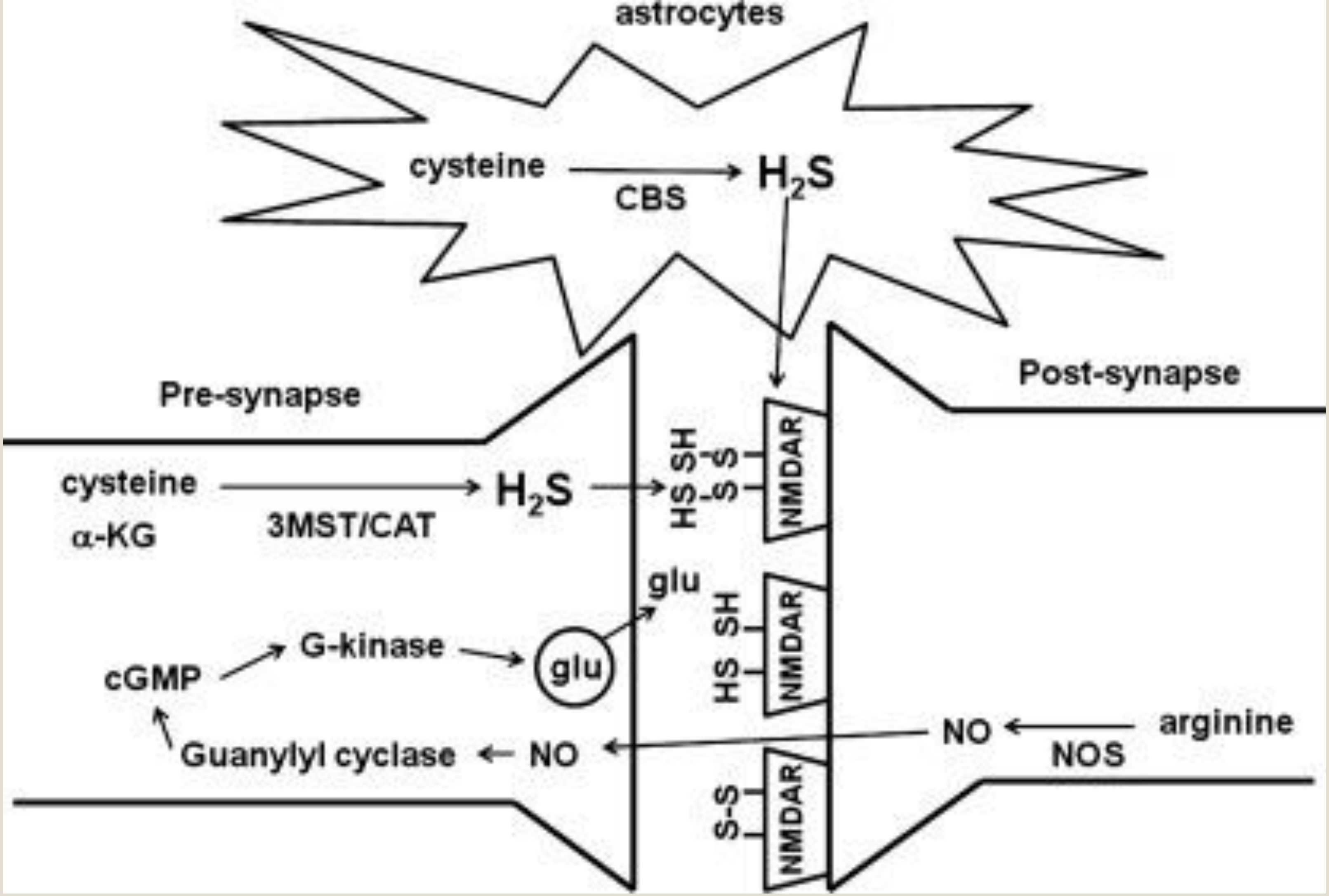
Hydrogen sulfide can also modulate inflammation, insulin release, and angiogenesis



H_2S can be produced by three enzymes,
cystathionine β-synthase (CBS),
cystathionine γ-lyase (CSE), and
3-mercaptopyruvate sulfurtransferase (3MST), along with cysteine aminotransferase (CAT), which is identical to aspartate aminotransferase



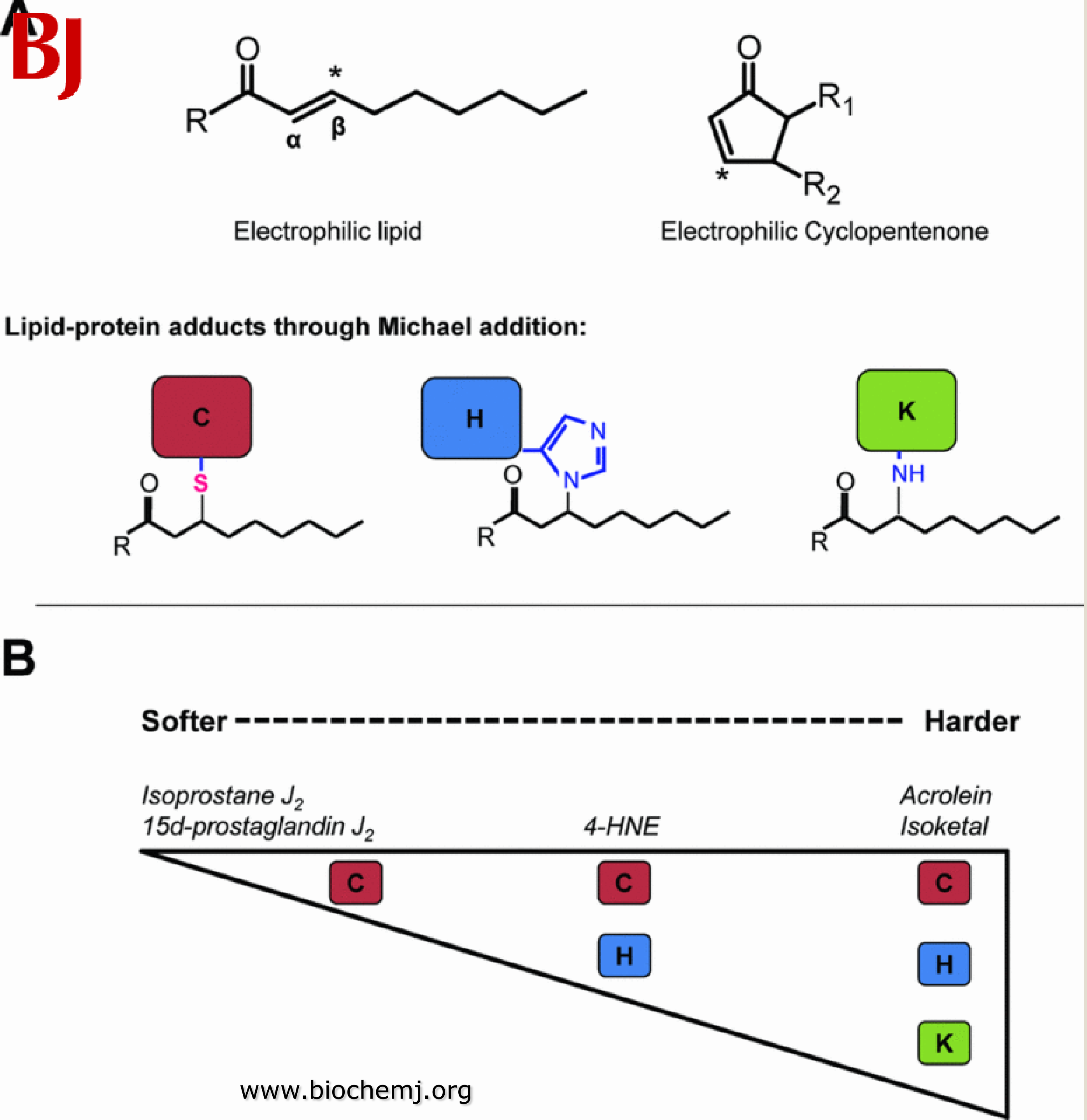
Modulation of enzyme activity by phosphorylation and sulfurhydration. The reversible incorporation of sulfur into proteins may modulate protein function as phosphorylation does on proteins. H_2S may directly induce sulfurhydration. Alternatively, H_2S may react with oxygen to form S^0 that adds to sulfhydryl groups of proteins.



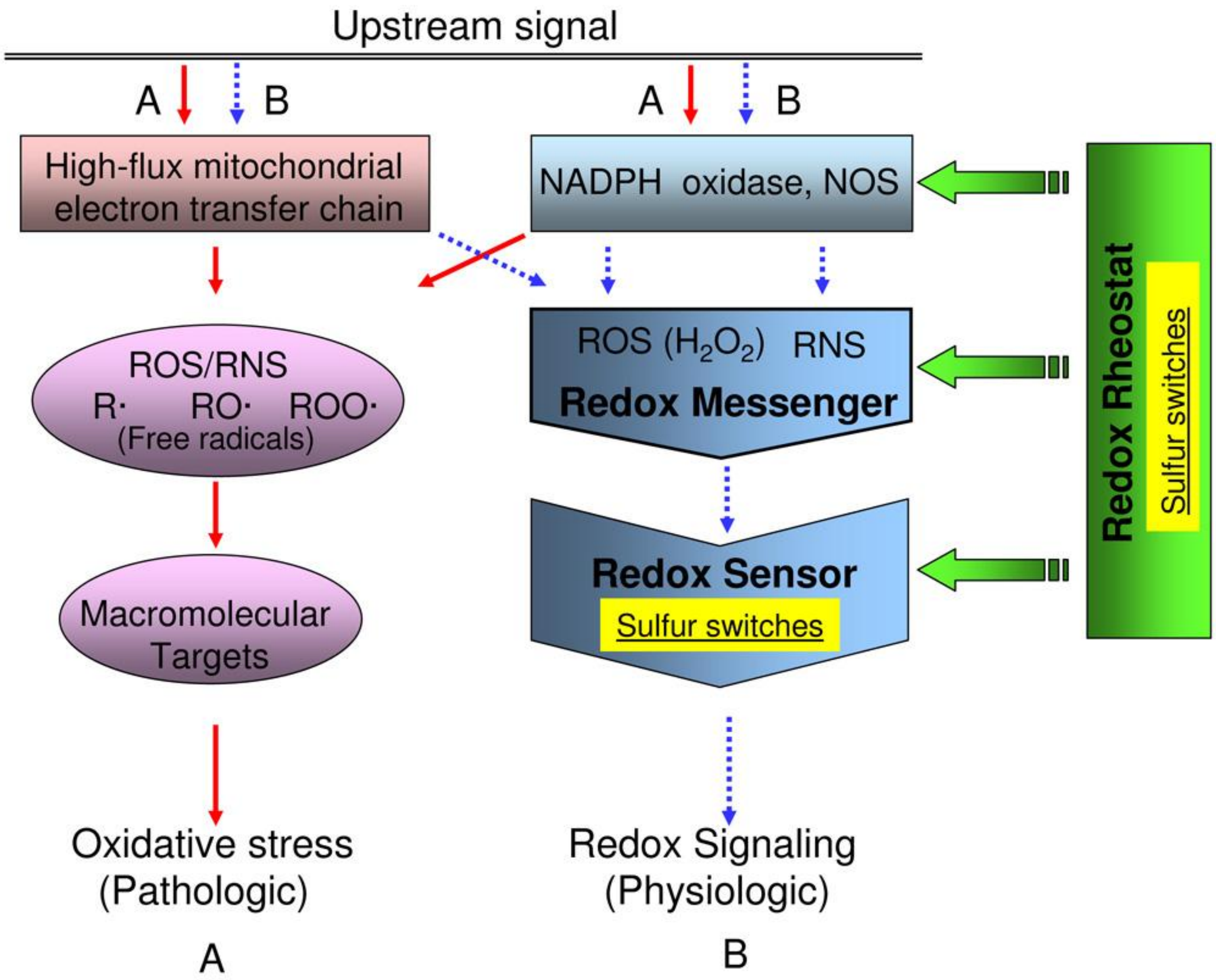
H_2S and nitric oxide (•NO) facilitate the induction of hippocampal long-term potentiation (LTP) with different mechanisms. •NO, which is produced from arginine, diffuses to presynapse to activate guanylyl cyclase, leading to the production of cyclic GMP that activates G-kinase to increase the release of a neurotransmitter glutamate. Although H_2S does not have any effect on guanylyl cyclase, it enhances the activity of NMDA receptors.

Redox signaling

Redox Lipid Species differ in their reactivity with specific amino acids


A
O
R
α
β
*
Electrophilic lipid
O
R1
R2
*
Electrophilic Cyclopentenone
Lipid-protein adducts through Michael addition:
C
S
O
R
H
N
N
O
R
K
NH
O
R
B
Softer ------------------------------------ Harder
Isoprostane J2
15d-prostaglandin J2
4-HNE
Acrolein
Isoketal
C
C
H
C
H
K

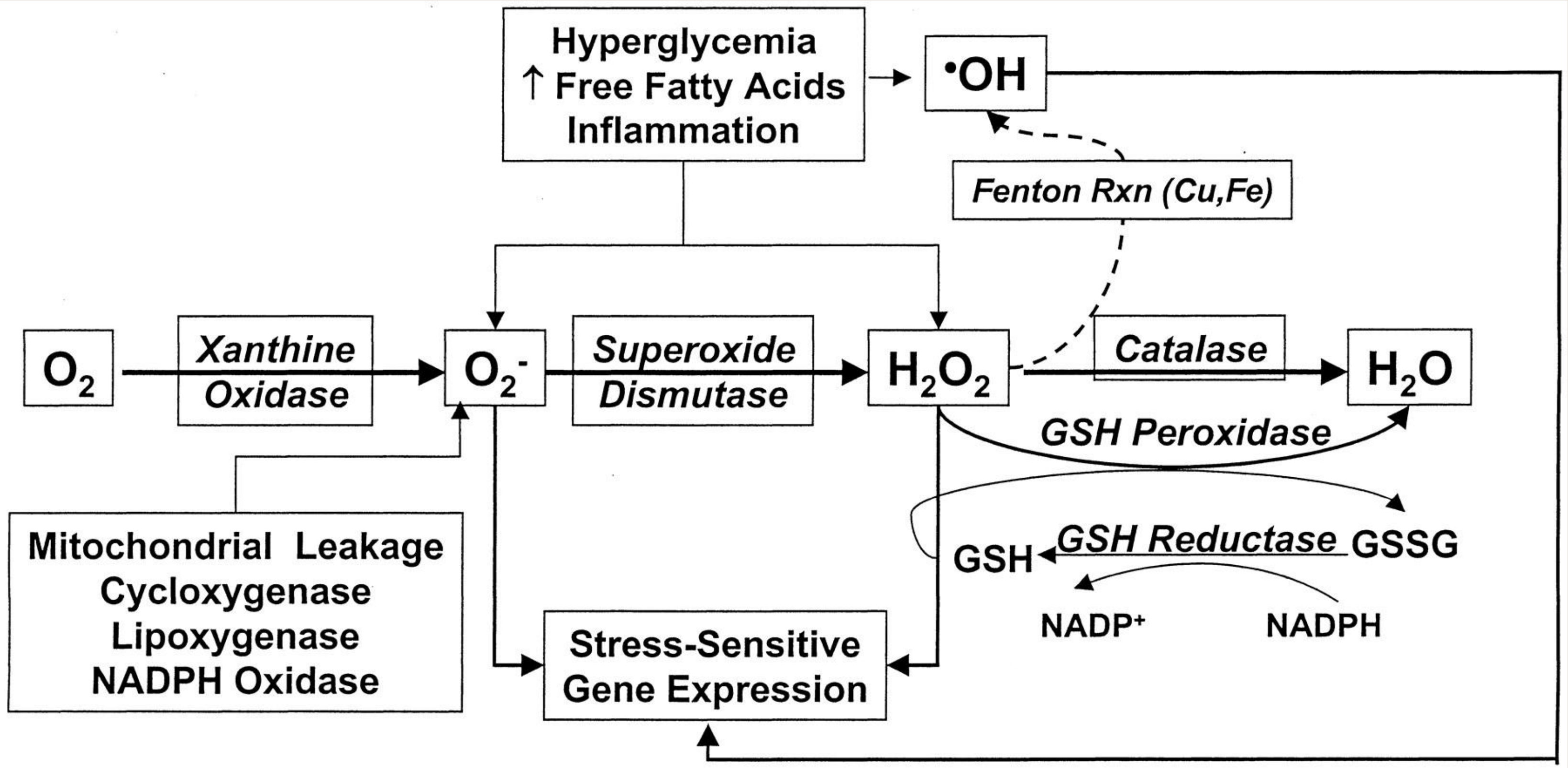
Biochemical Journal 2012 442, 453-464 - Ashlee Higdon, Anne R. Diers and others.



Sulfur switches can exist as "Redox Sensors" and as "Redox Rheostats" in generic redox circuitry models. Simplified models show (A) oxidative stress pathway (solid line) associated with high levels of ROS, RNS, and free radicals from mitochondrial dysfunction, NADPH oxidase activation, and nitric oxide synthase activation triggered by pathologic upstream signals.

Redox signaling pathway (B, broken line) shows the process involving relatively low levels of diffusible reactive species associated with physiologic condition. In redox signaling, the small diffusible reactive species, termed "redox messengers" are generated by ROS/RNS sources and react with redox-sensitive macromolecules termed "redox sensors".

Exogenous and endogenous stimuli leading to ROS generation and activation of stress-sensitive gene expression.



Evans J L et al. Endocrine Reviews 2002;23:599-622

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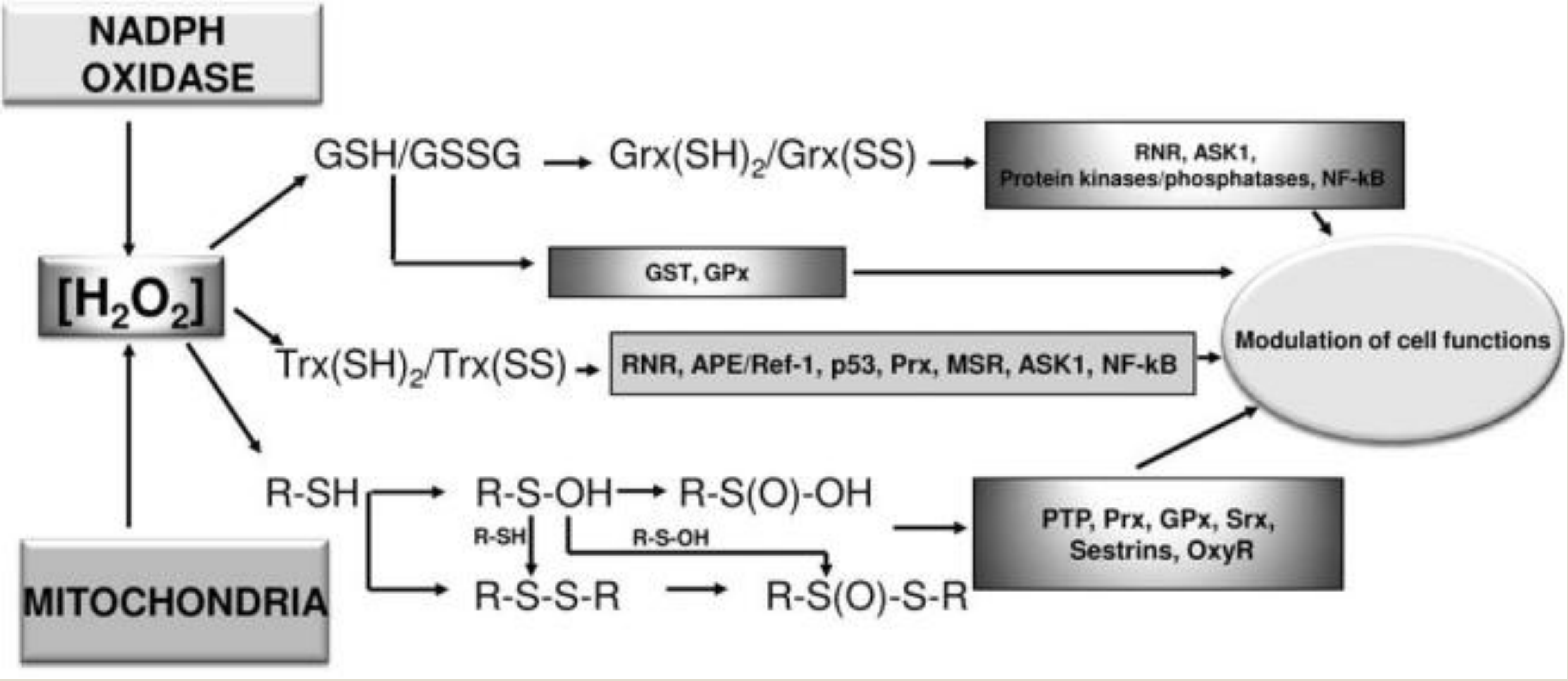
Signaling mediated by hydrogen peroxide



NADPH OXIDASE

MITOCHONDRIA

$[H_2O_2]$

GSH/GSSG → $Grx(SH)_2/Grx(SS)$ →

RNR, ASK1,
Protein kinases/phosphatases, NF-kB

GST, GPx

$Trx(SH)_2/Trx(SS)$ →

RNR, APE/Ref-1, p53, Prx, MSR, ASK1, NF-kB

R-SH

R-S-OH → R-S(O)-OH

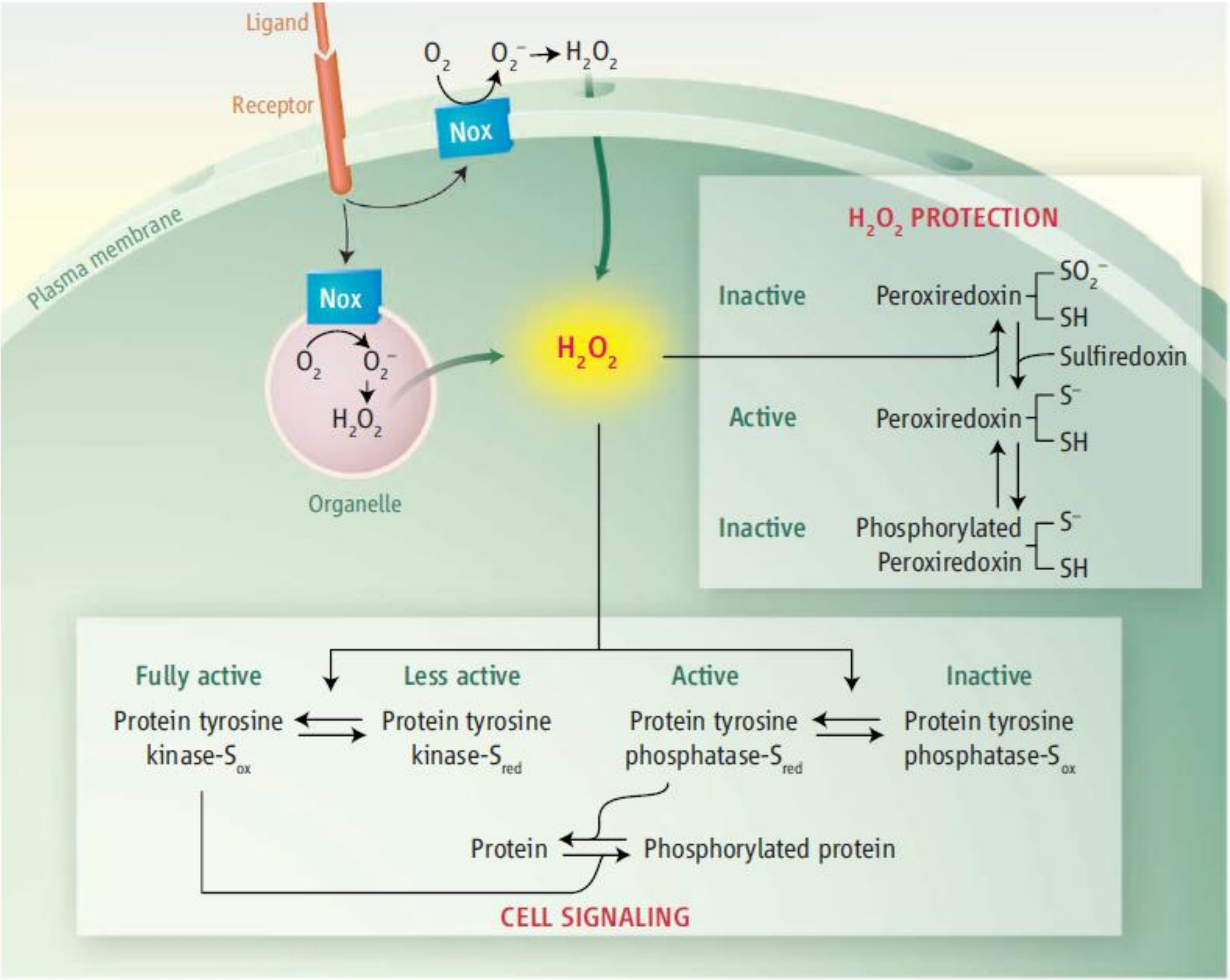
R-SH

R-S-OH

R-S-S-R → R-S(O)-S-R

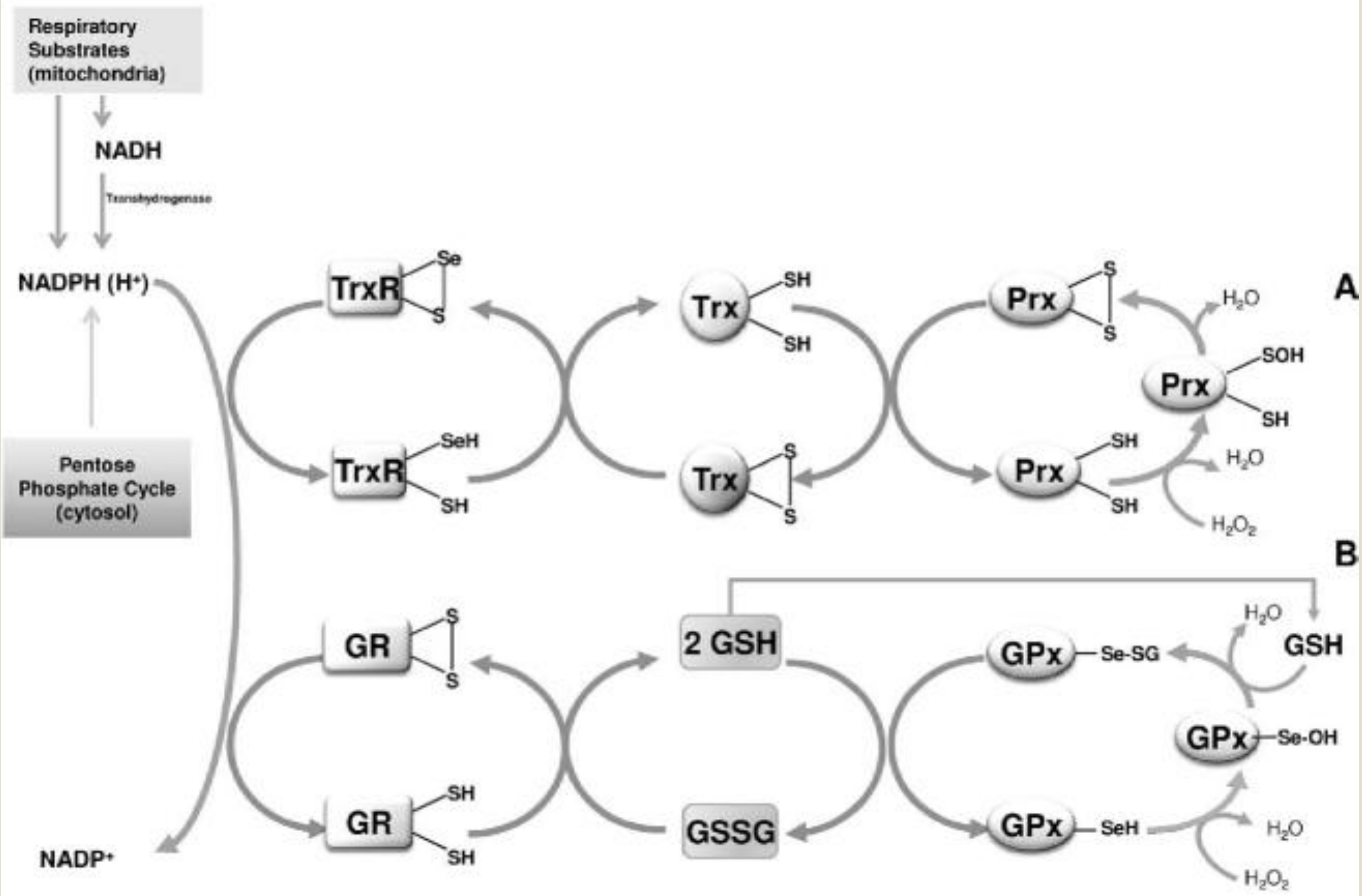
PTP, Prx, GPx, Srx,
Sestrins, OxyR

Modulation of cell functions

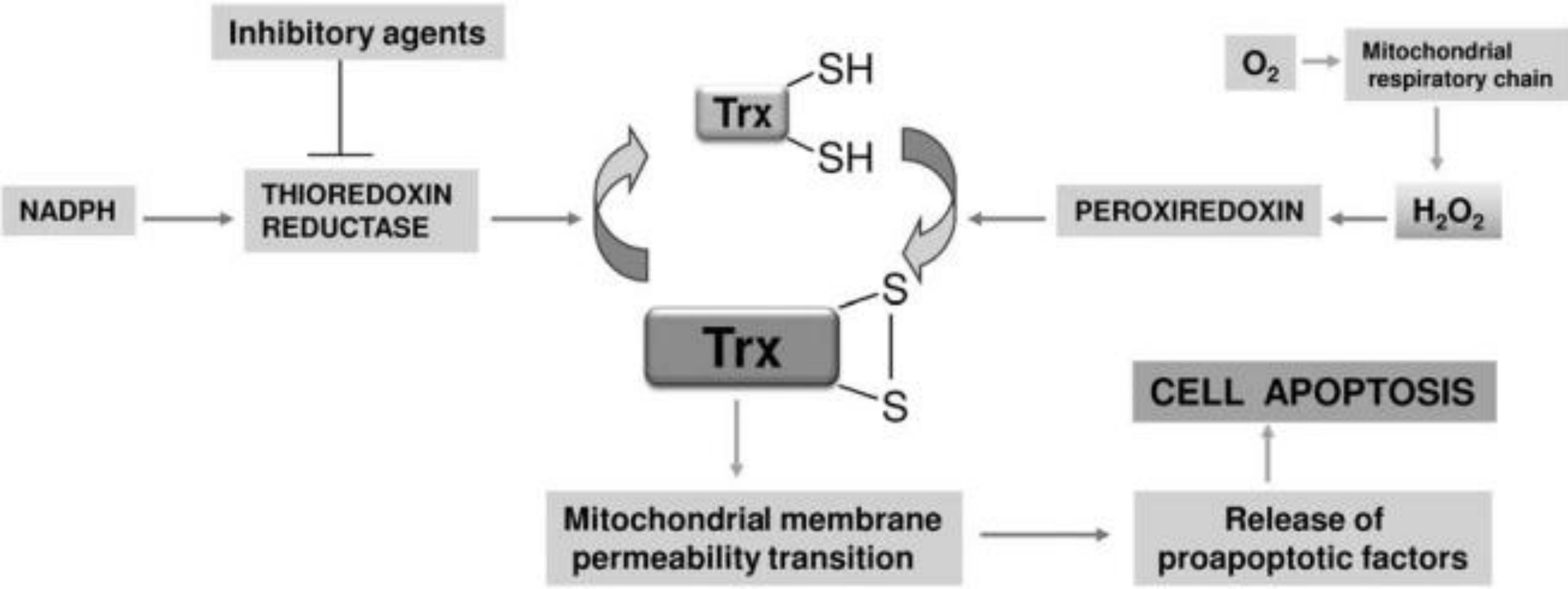


H_2O_2 production, protection, and signaling actions. Activation of various cell surface receptors activates Nox

Reduction of hydrogen peroxide mediated by thioredoxin (A) and glutathione (B) pathways.



Mitochondrial alterations due to thioredoxin reductase inhibition



Inhibitory agents

NADPH

THIOREDOXIN REDUCTASE

Trx

SH

SH

Trx

S

S

O_2

Mitochondrial respiratory chain

H_2O_2

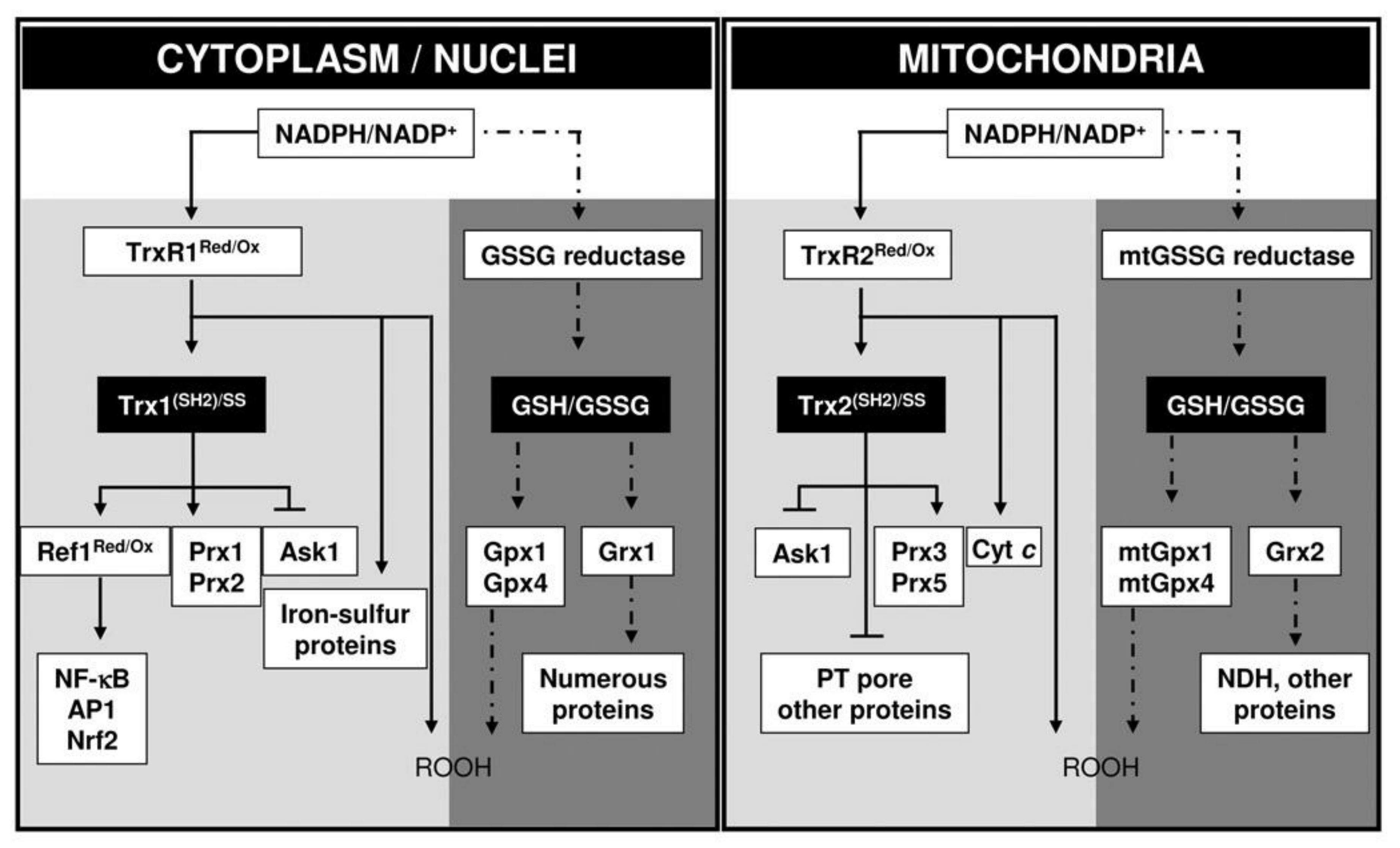
PEROXIREDOXIN

CELL APOPTOSIS

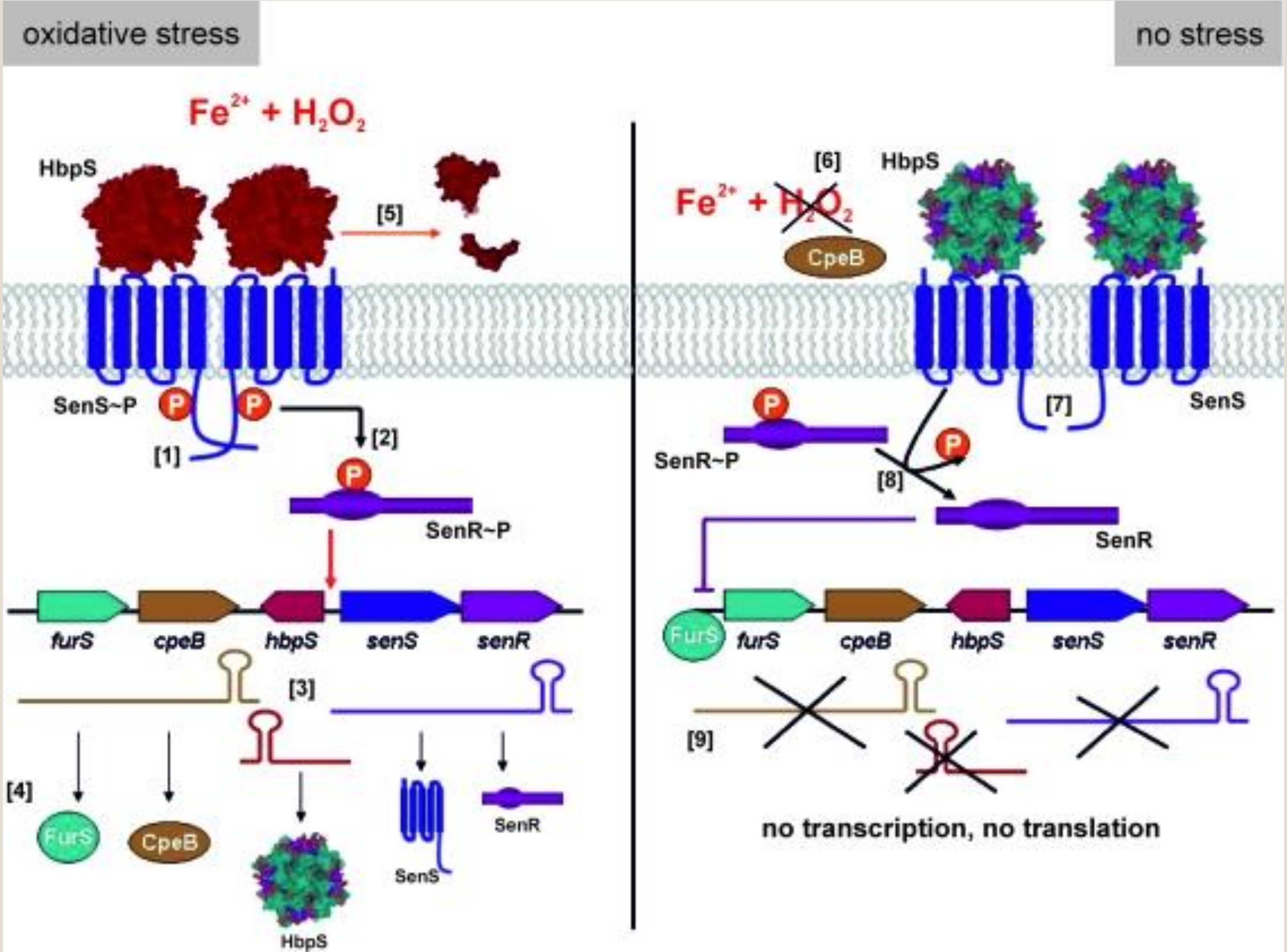
Mitochondrial membrane permeability transition

Release of proapoptotic factors

GSH and Trx-dependent systems support different protein-dependent systems in cytoplasm and nuclei (left), and in mitochondria (right).

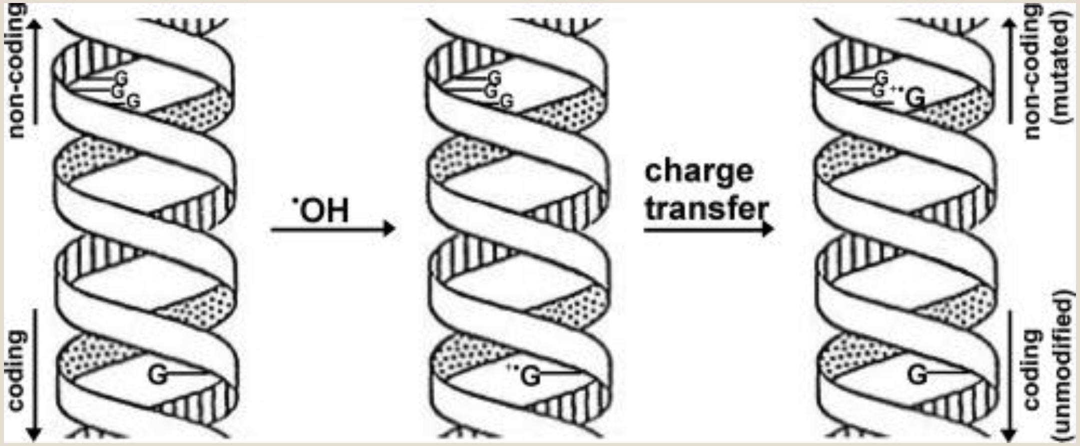


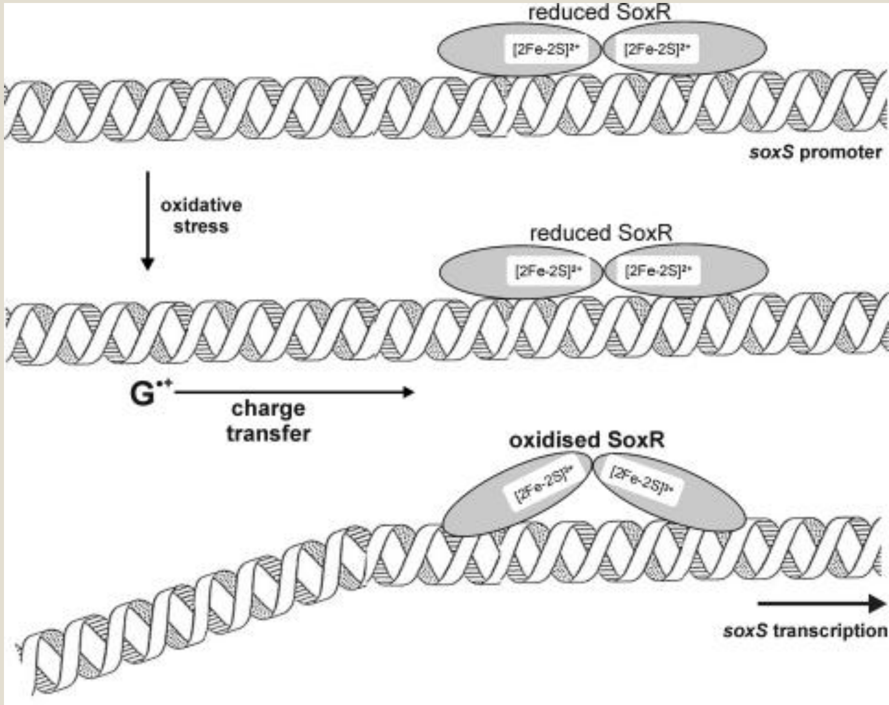
Schematic view of the mode of action of the redox-sensing system HbpS-SenS-SenR



- Schematic view of the mode of action of the redox-sensing system HbpS-SenS-SenR. Under iron-mediated oxidative-stressing conditions (left) the oxidized and structure-changed form of HbpS (red) activates the autophosphorylation of the sensor kinase SenS, leading SenS∼P [1]. SenS∼P phosphorylates the response regulator SenR, leading SenR∼P [2]. This activates the transcription of hbpS and senS-senR, and de-represses the transcription of furS-cpeB [3]. Consequently, FurS, CpeB, HbpS, SenS, and SenR are produced [4] and participate in the antioxidative stress response. While SenS, CpeB, and HbpS are transported into the membrane (SenS) or extracellular space (CpeB and HbpS), FurS and SenR remain cytoplasmic. The oxidized and structure-changed form of HbpS undergoes a time-dependent degradation during oxidative stress [5]. Once CpeB associates with the mycelia, it degrades H2O2 providing S. reticuli with a nonoxidized environment and blocking the oxidation of HbpS [6] (right). Freshly synthesized and unmodified HbpS (mixed color, turquoise, and green) interacts with SenS and inhibits its autophosphorylation [7]. SenS in turn dephosphorylates SenR∼P, leading SenR [8]. This, together with FurS, represses the transcription of furS-cpeB and de-activates the transcription of hbpS and senS-senR [9].

Charge transfer in DNA





A model showing DNA-mediated activation of SoxR

Parameters of signal transduction

1. **Signal molecules**: properties (hydrophobicity) → location of R (surface/ intracellular)
2. **Properties of receptors** (R): specificity to signal (type of signal → type of R); ability to transduce the signal (binding/ catalytic); adaptation of R to signal (desensitization; feedback regulation)
3. **Signaling pathway**: structure of adaptors; amplification of signal (2 strategies depending on surface/intracellular location of R); integration of signal pathways (cross-talk; coordination of response)
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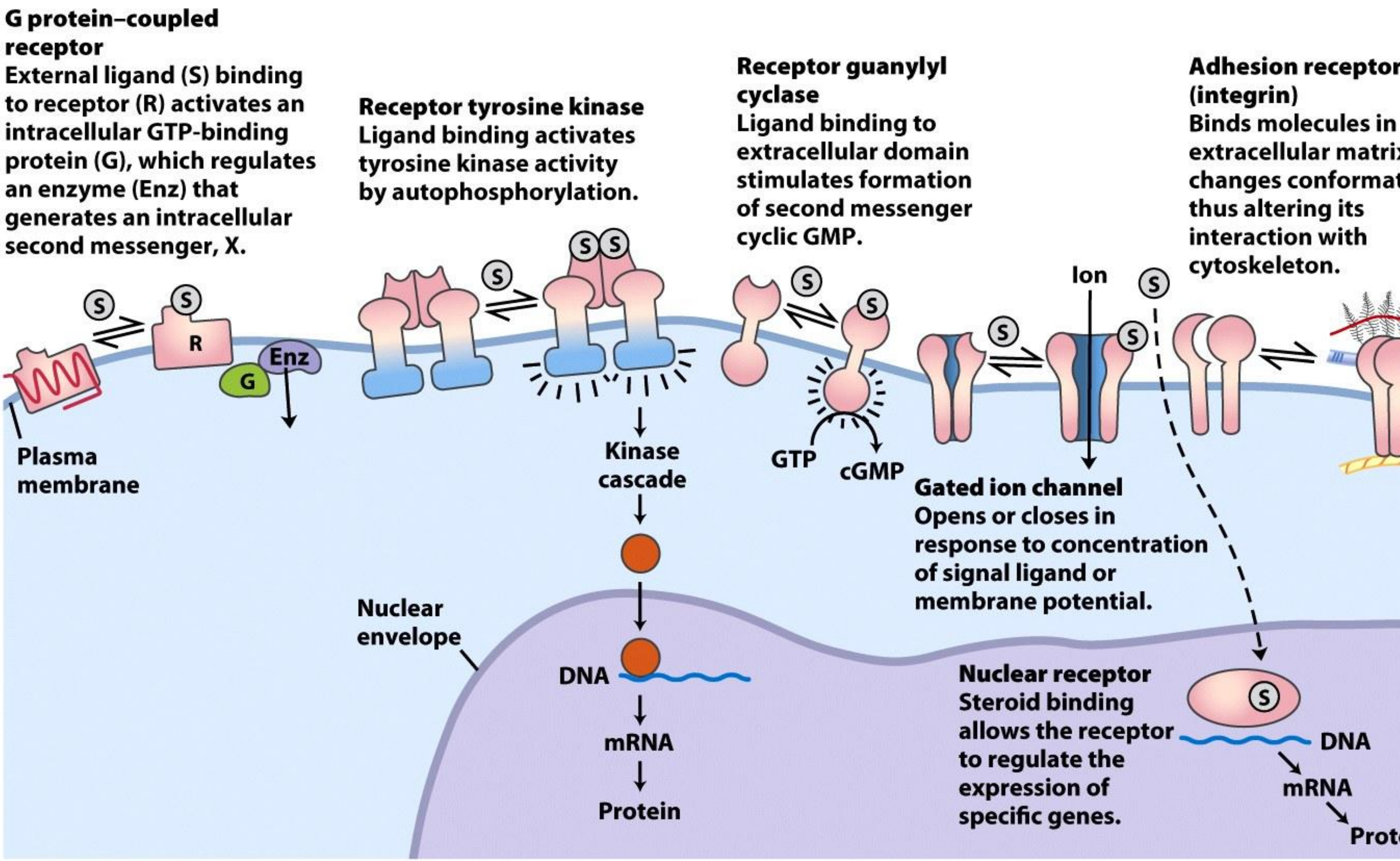
5. For multicellular organisms: **hierarchy of signal systems**; location-, dose- and time-dependent effects



G protein–coupled receptor
External ligand (S) binding to receptor (R) activates an intracellular GTP-binding protein (G), which regulates an enzyme (Enz) that generates an intracellular second messenger, X.

Receptor tyrosine kinase
Ligand binding activates tyrosine kinase activity by autophosphorylation.

Receptor guanylyl cyclase
Ligand binding to extracellular domain stimulates formation of second messenger cyclic GMP.

Adhesion receptor
(integrin)
Binds molecules in extracellular matri
changes conformat
thus altering its interaction with cytoskeleton.

Gated ion channel
Opens or closes in response to concentration of signal ligand or membrane potential.

Nuclear receptor
Steroid binding allows the receptor to regulate the expression of specific genes.

Figure 12-2
Lehninger Principles of Biochemistry, Fifth Edition

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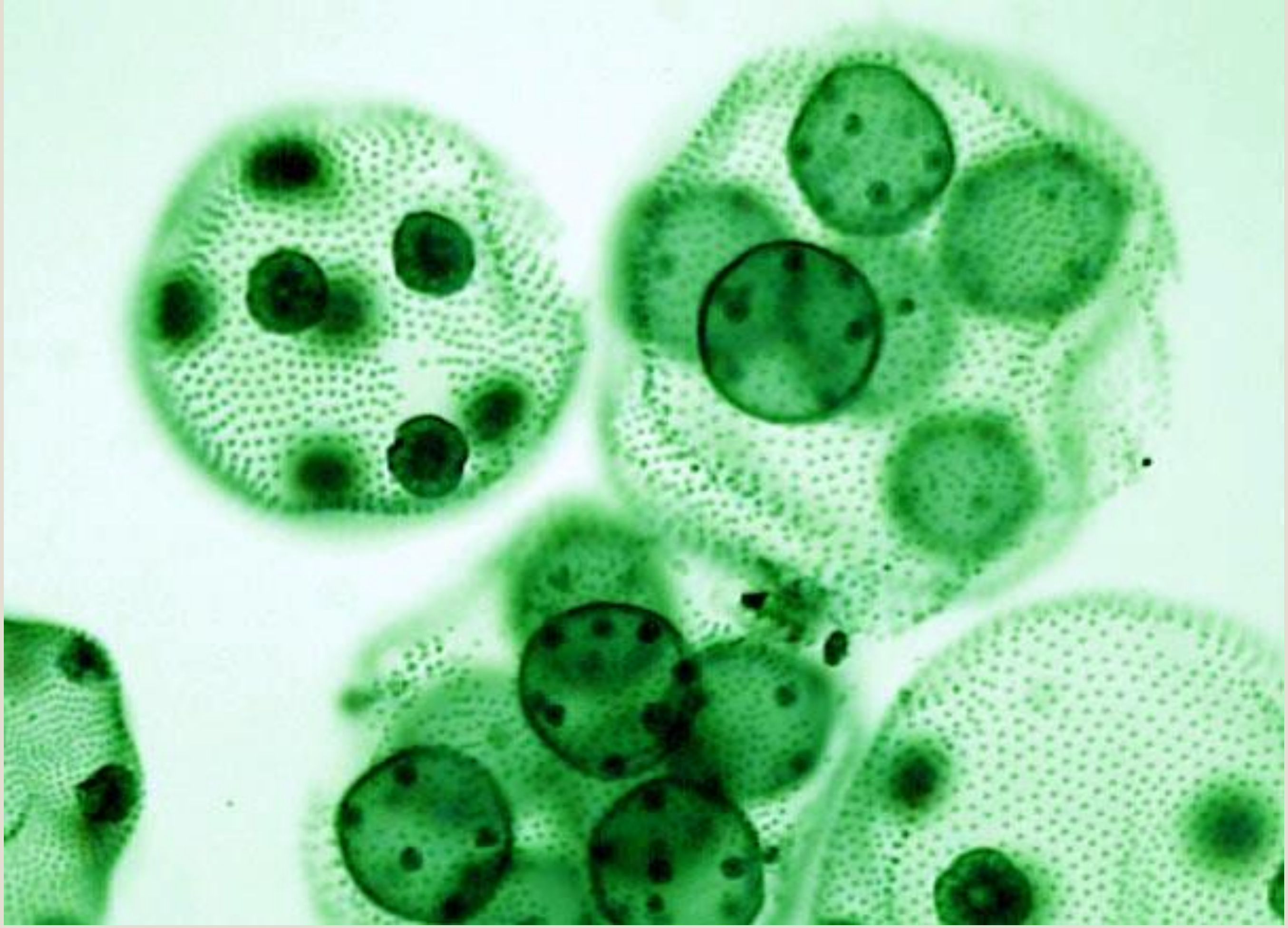
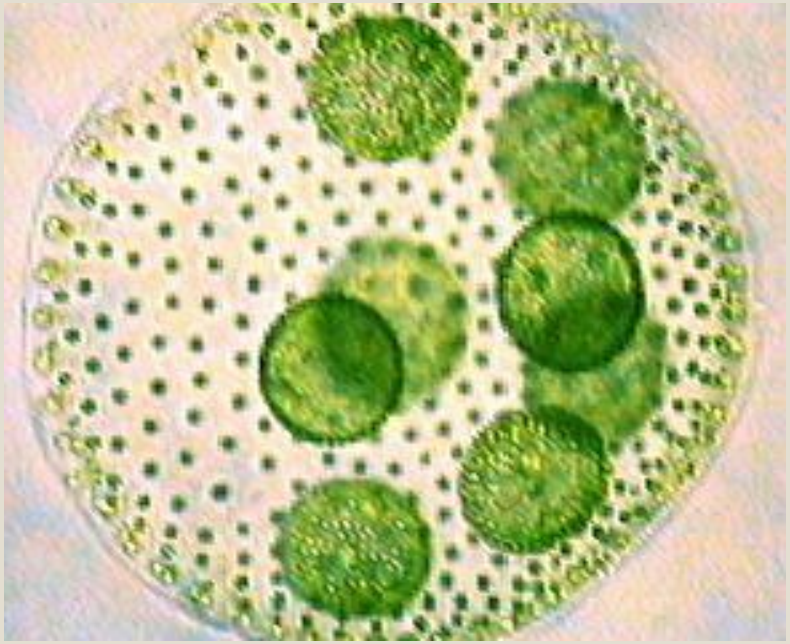
Cell-Cell Interactions



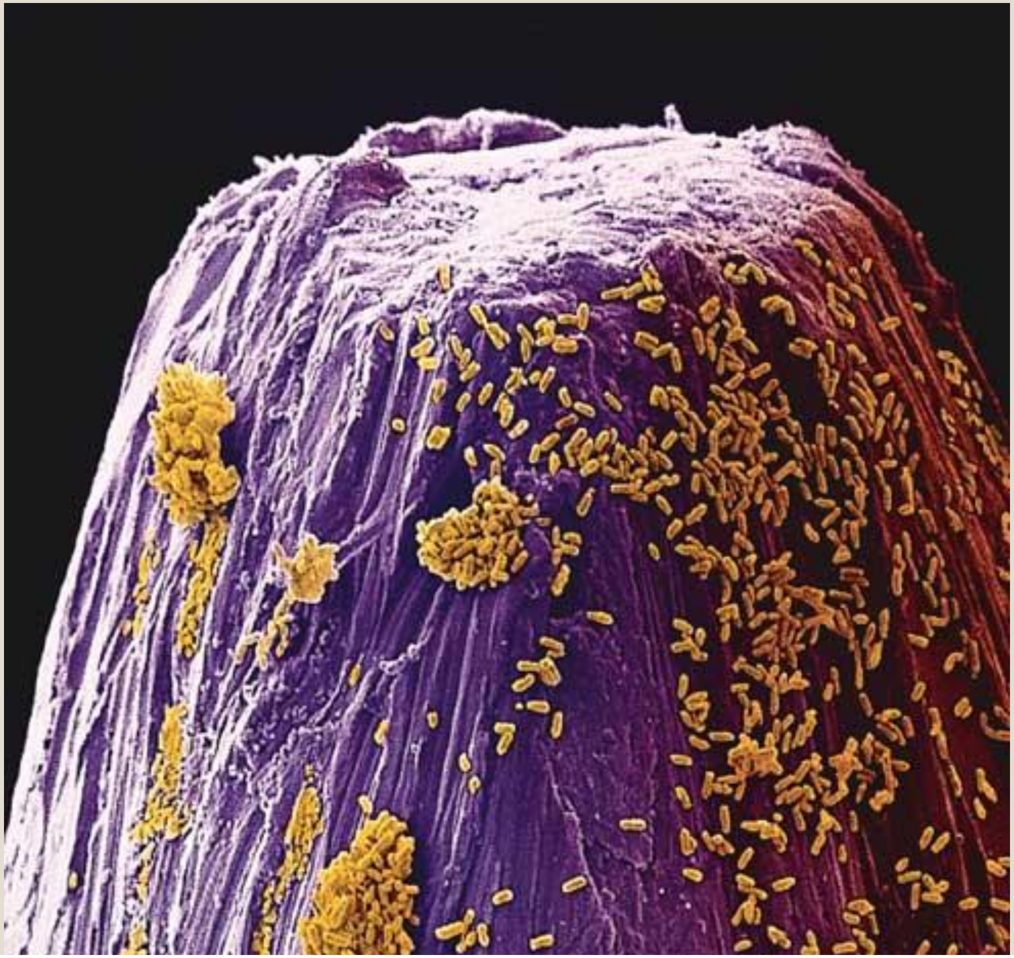
Multicellular Organisms Require Cell-Cell Interactions

Evolution of multicellularity

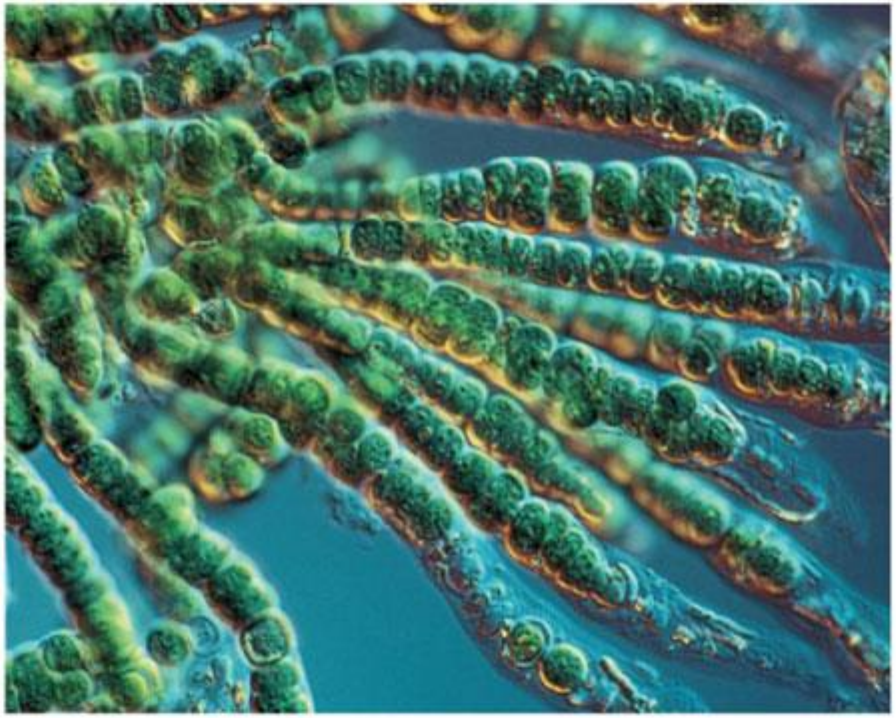


Volvox, an algae

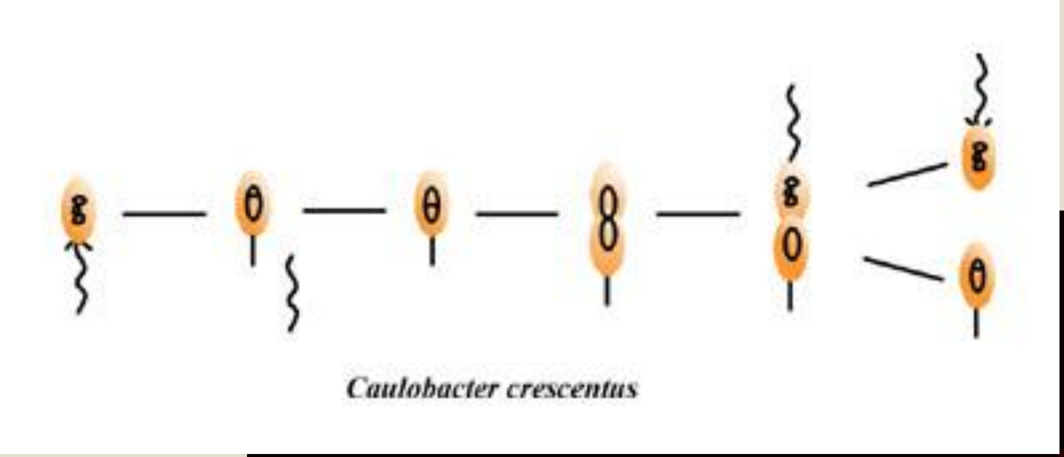
Cell-Cell Adhesion Defines Multicellularity



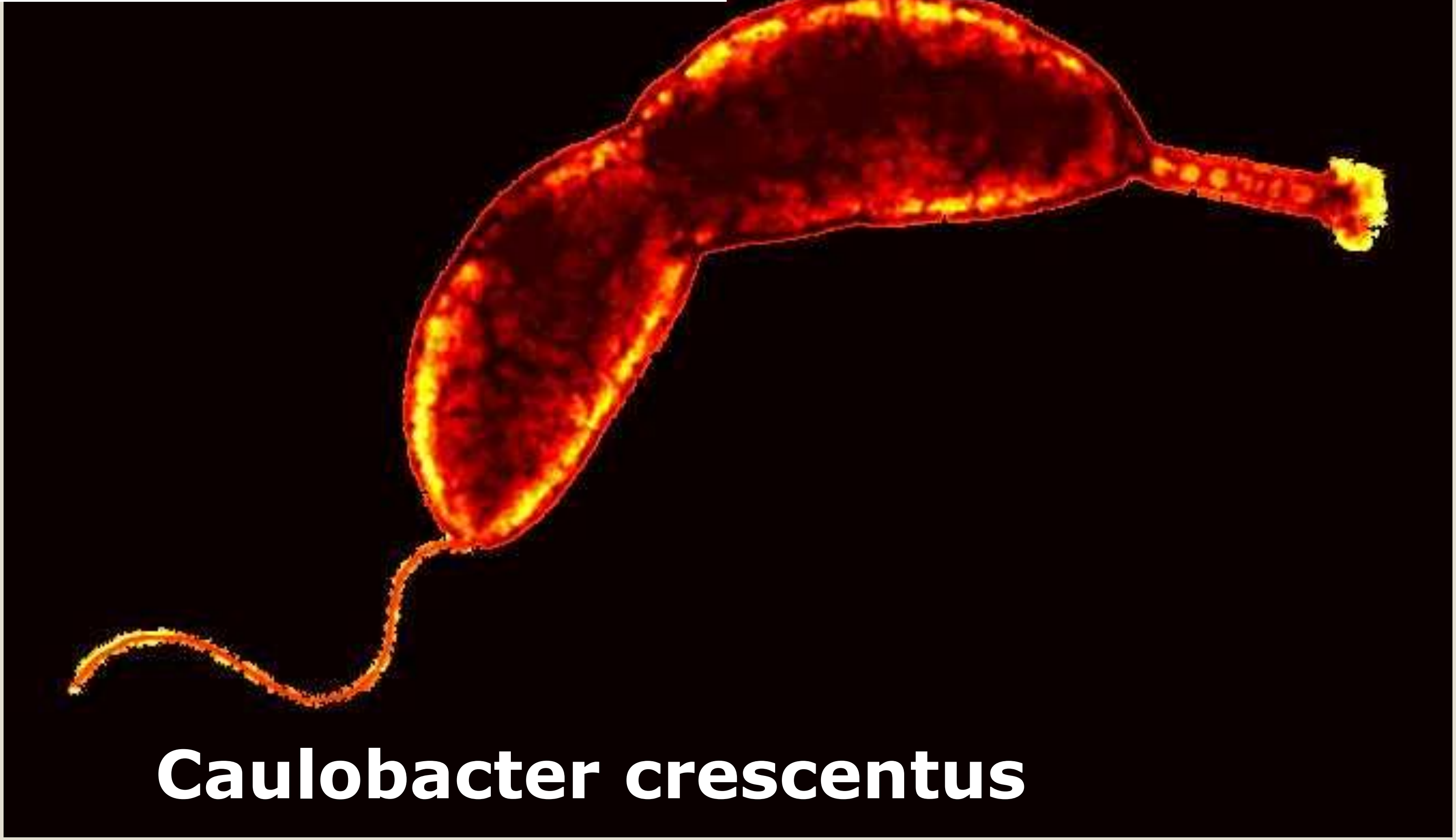
The first traces of life appear nearly 3.5 billion years ago, in the early Archaean. Clearly identifiable fossils remain rare until the late Archaean, when stromatolites, layered mounds produced by the growth of microbial mats, become common in the rock record.



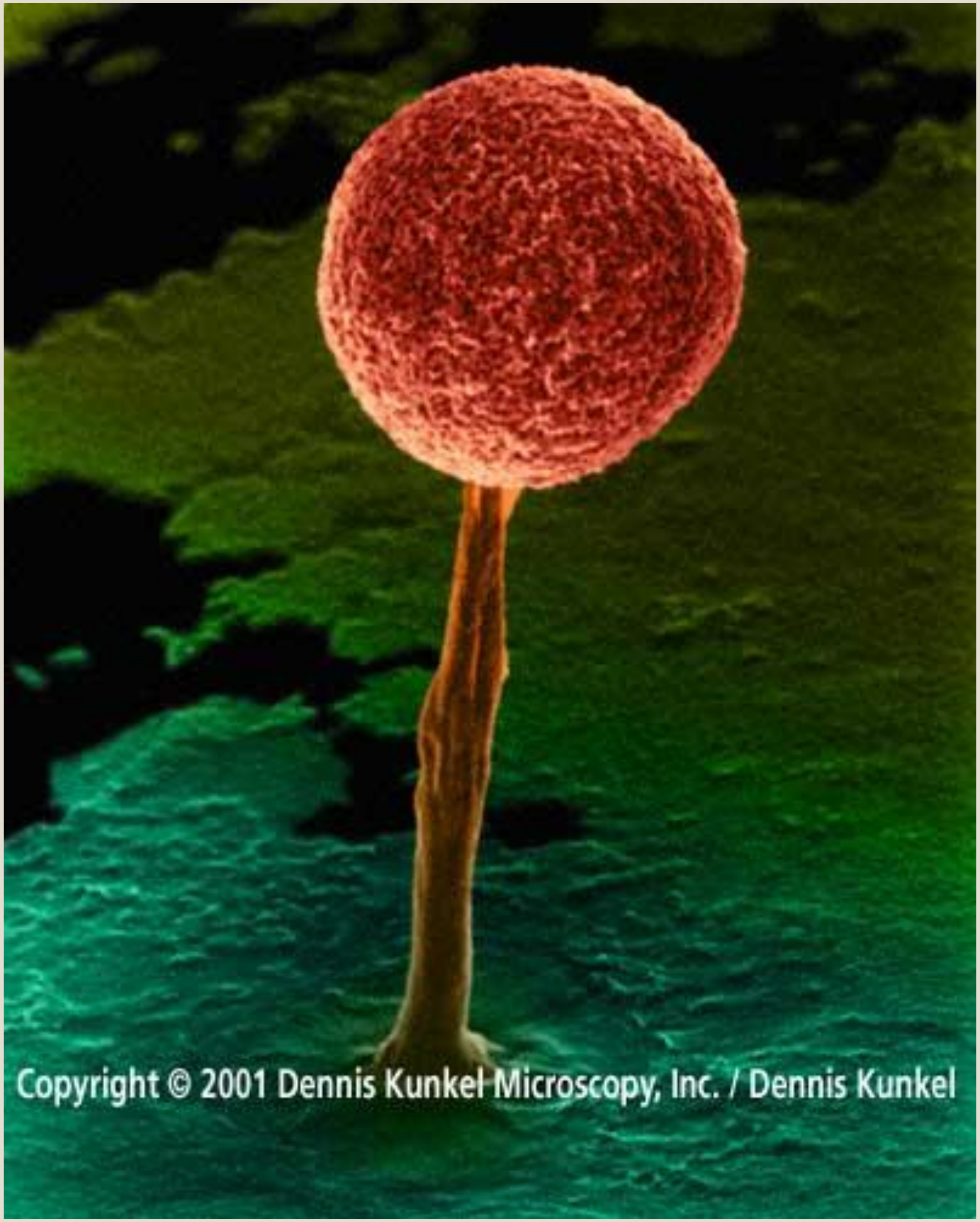
Cyanobacteria



Caulobacter crescentus



Caulobacter crescentus



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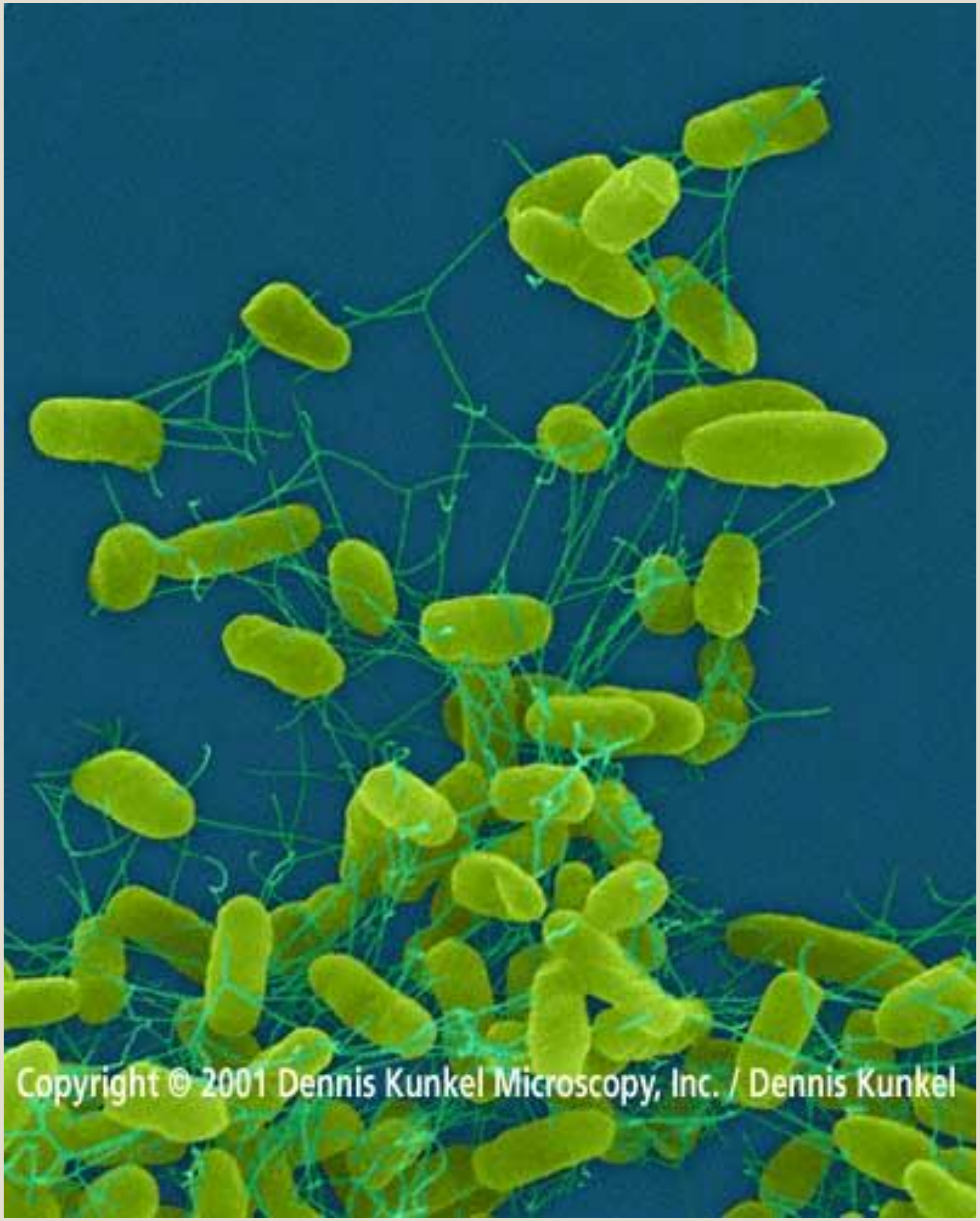
More Complex Multicellular Organisms have Specialized Cell Types

Cells Can Interact With Each Other Indirectly Through Protein-based Scaffolds



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Bacteria cells communication

Biofilms – Now a Universal Feature

- Now scientists believe biofilm formation is a universal feature of all bacteria

Microbial Mats = Biofilms

Attachment of a bacterium to a surface, or substratum, is the initial step in the formation of a biofilm

Cell-surface adhesins that mediate contact with the host matrix

The maturation of a biofilm community and their architecture can vary from flat, homogenous biofilms, to highly structured biofilms,

connection between quorum sensing and biofilm formation

Bacterial and fungal pathogens form biofilms

Biofilms – Now a Universal Feature

- Now scientists believe biofilm formation is a universal feature of all bacteria

Biofilms

- Most bacteria exist in their natural habitats as sessile **multicellular communities** called **biofilms** in which the bacterial population differentiates as part of the development process (Costerton et al., 1995; Vlamakis et al., 2008). Most bacterial species can live and grow within a shared habitat, and co-ordinate **complex group behaviour** that benefits the whole community (Shapiro, 1998).
- Examples of such behaviour leading to the propagation of bacterial spores include
(1) fruiting body formation in Myxococcus xanthus (Kaplan, 2003),
(2) biofilm formation and fruiting-body-like assembly in Bacillus subtilis (Branda et al., 2001) and
(3) aerial hyphae development in Streptomyces coelicolor (Claessen et al., 2006).

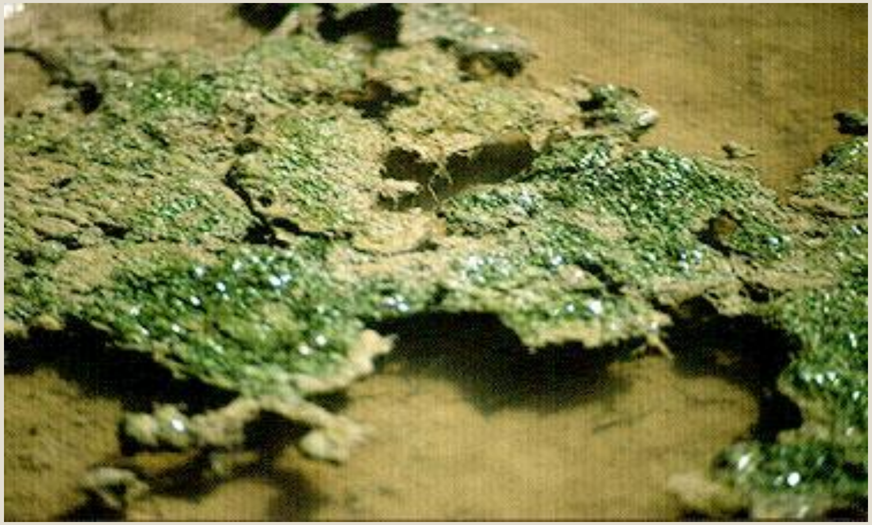
Microbiology (2009), 155, 1–8

Biofilms are multicellular communities held together by a self-produced **extracellular matrix**. The mechanisms that different bacteria employ to form biofilms vary, frequently depending on environmental conditions and specific strain attributes

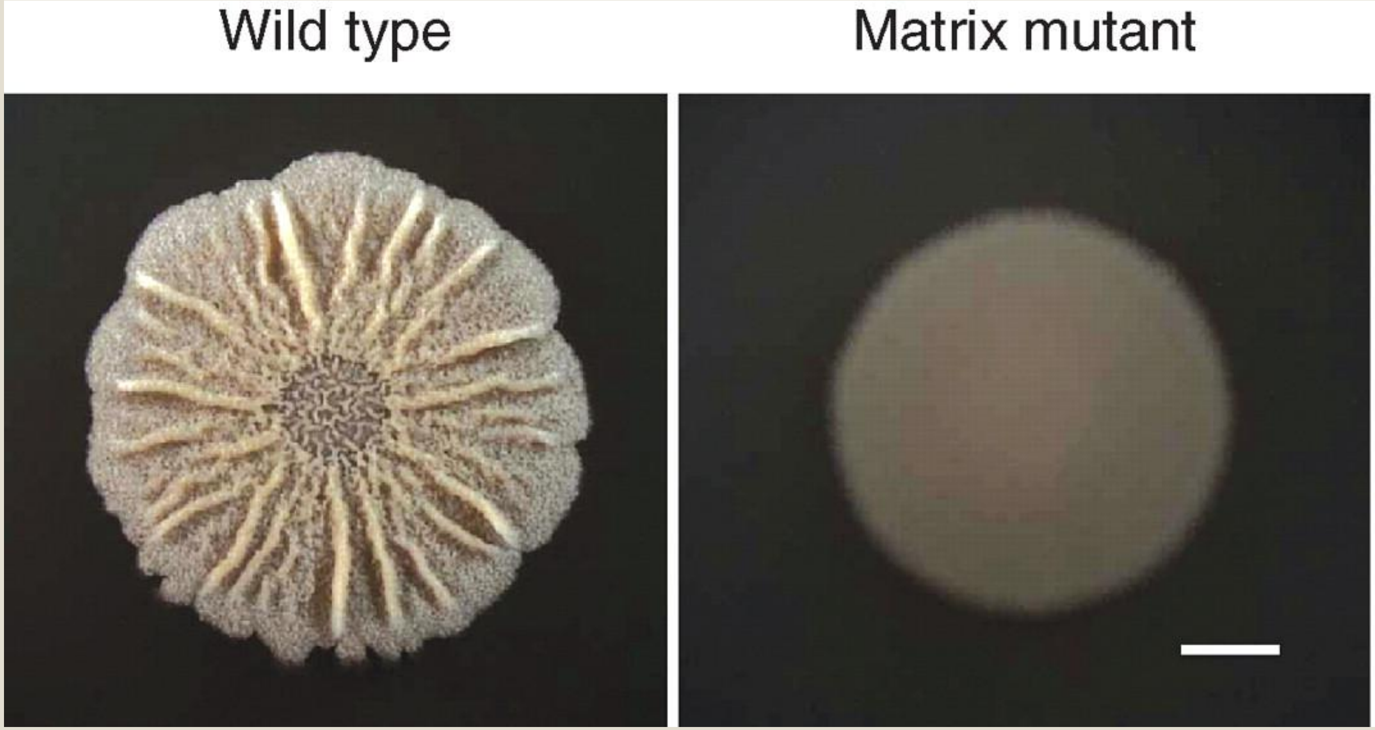
Cold Spring Harb Perspect Biol 2010

Formation of biofilms in nature

- Biofilms offer their member cells several benefits
- Biofilms are diverse from their formation on teeth as plaques and submerged rocks in a stream



Colony morphology of B. subtilis strain 3610 wild type and matrix mutant (eps).



López D et al. Cold Spring Harb Perspect Biol 2010;2:a000398

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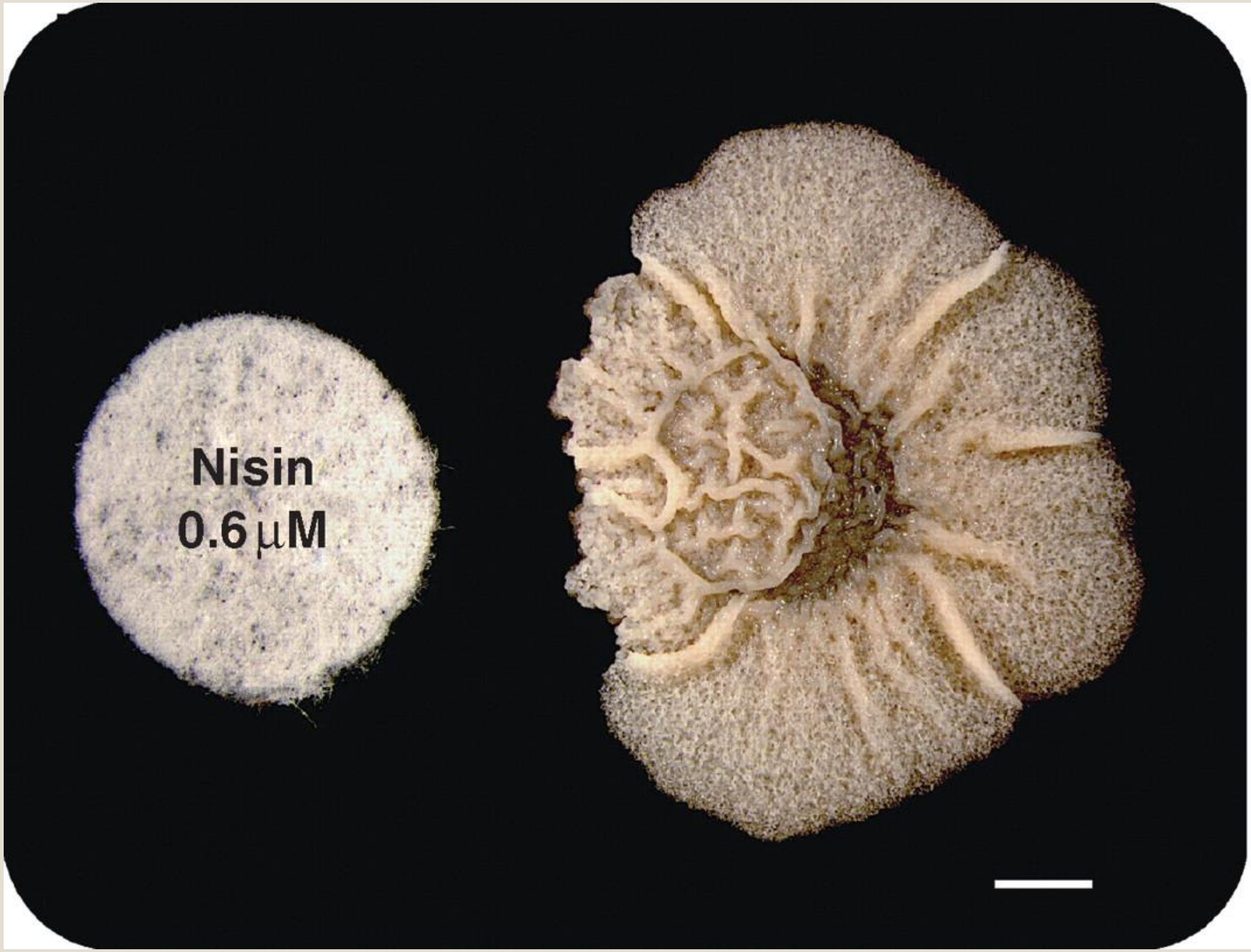
Biofilm characteristics

- Submerged biofilms seems to form **columns and mushroom** like projections that are separated by water-filled channels
- Floating biofilms form a skin or pellicle at the air-liquid interface – shows organization of cells with the matrix at the outside
- Films that form on the surface of solid media such as agar or other surfaces

Effect of the antimicrobial nisin on B. subtilis biofilm morphology.



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2010;2:a000398**

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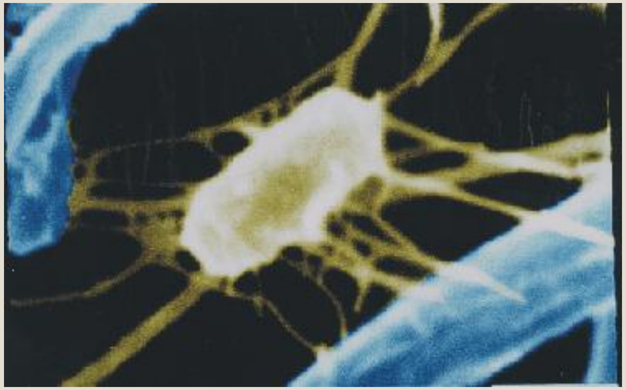
Biofilms

BIOFILMS may form:

- On solid substratums in contact with moisture
- On soft tissue surfaces in living organisms
- At liquid-air interfaces.

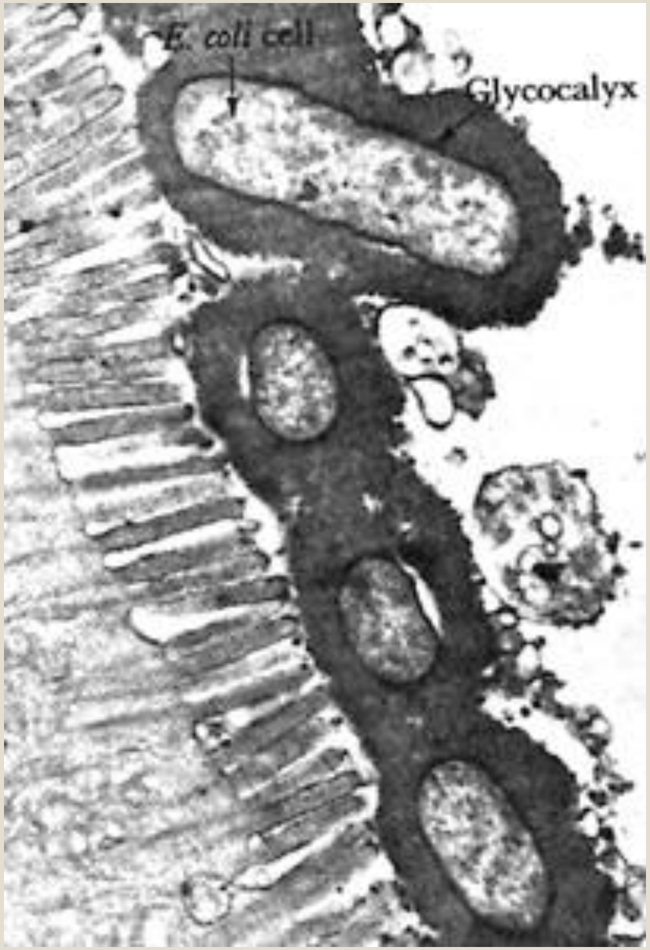
Matrix

- Key components of the matrix are extracellular polysaccharides and proteins
- Dead cells have also been identified in biofilms
- Extracellular DNA is also important



Exopolysaccharides

- Exopolysaccharides
- In the glycocalyx contribute to biofilm formation



Polysaccahrides

- Many bacteria **have been found to produce cellulose**
- This is a novel finding in the case of *Salmonella typhimurium* and *E. coli*
- The bacterium *Gluconacetobacter xylinus* has been recognized as a cellulose producer
- Many other bacteria have genes homologous to the bcs, bacterial cellulose synthesis genes
- *Vibrio cholera* does not appear to have a gene which encodes a cellulose – but the bacterium has two domains homologous to *Gluconeacetobacter*.

Pseudomonas aeruginosa

- *Pseudomonas* strains were thought to produce **alginate**
- However alg mutants did not produce changes in the biofilm formation
- Two new polymers have been found in *Pseudomonas* polymers
- pelA-G gene produces a glucose rich polymer
- pslA-O genes produce a mannose rich polymer

Biofilm Genetics - initiation

- Staphylococcal **polysaccharide intercellular adhesin (PIA)**
- PIA – poly-N-acetyl glucosamine (PNAG) polymer
- PIA polymers present in *E. coli*
- **The pga gene is involved with the formation of biofilms**
- This gene is similar to the staphylococcal gene ica
- Now *Yersinia pestis* has been shown to make a gene homologous to this

Bap Protein

- The biofilm associated protein
- Structurally similiar to the surface proteins

Esp of *Enterococcus faecalis*

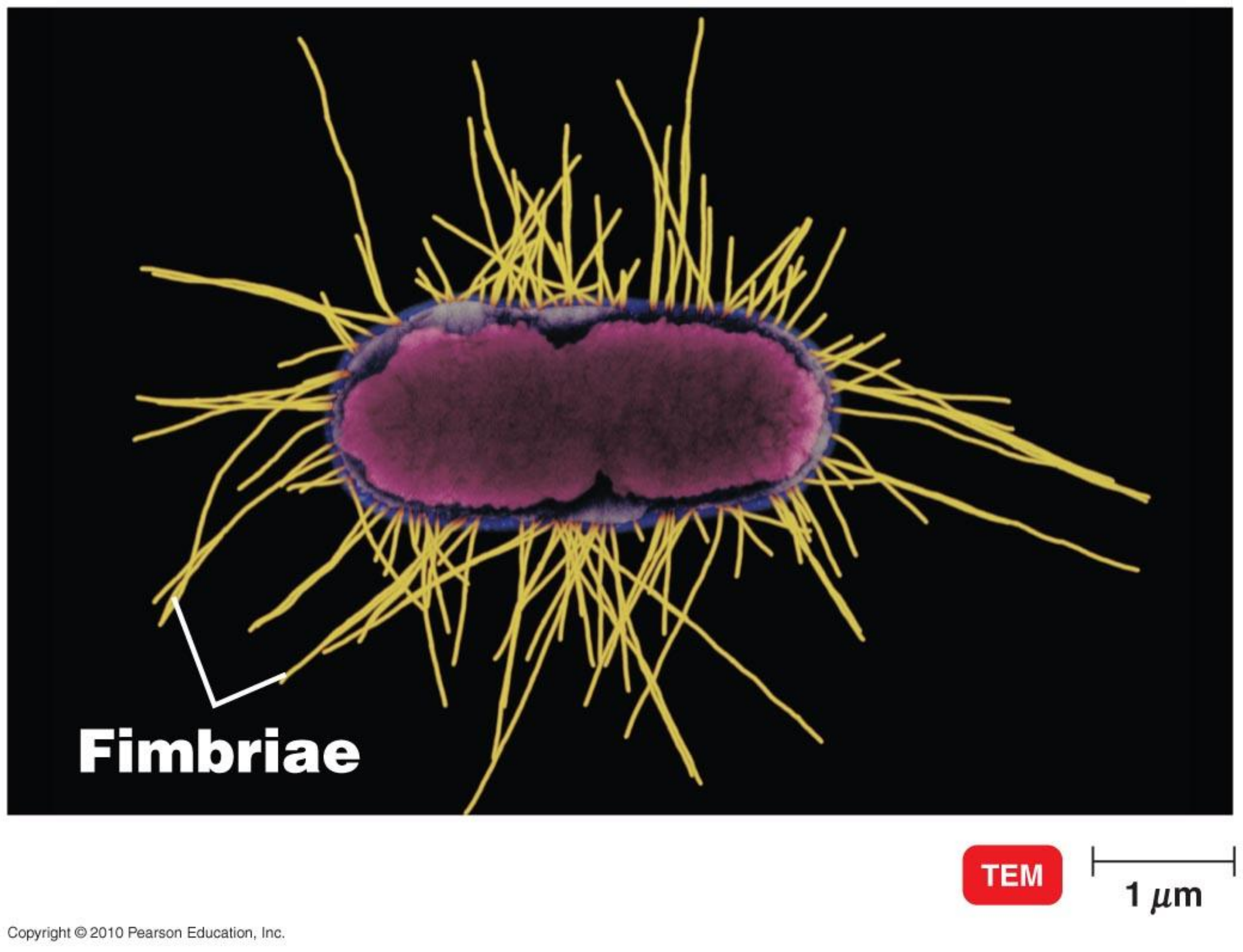
mus20 of *Pseudomonas aeruginosa*

sty2875 of *Salmonella typhi*

Conjugative pili

- Conjugative **pili greatly accelerates initial adhesion and biofilm development** by *E. coli*
- **Gram negative bacteria have adhesins** at the tip of its fimbriae
- *E. coli* responds to levels of nutrients and osmolarity

Fimbriae and Pili



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Fimbriae

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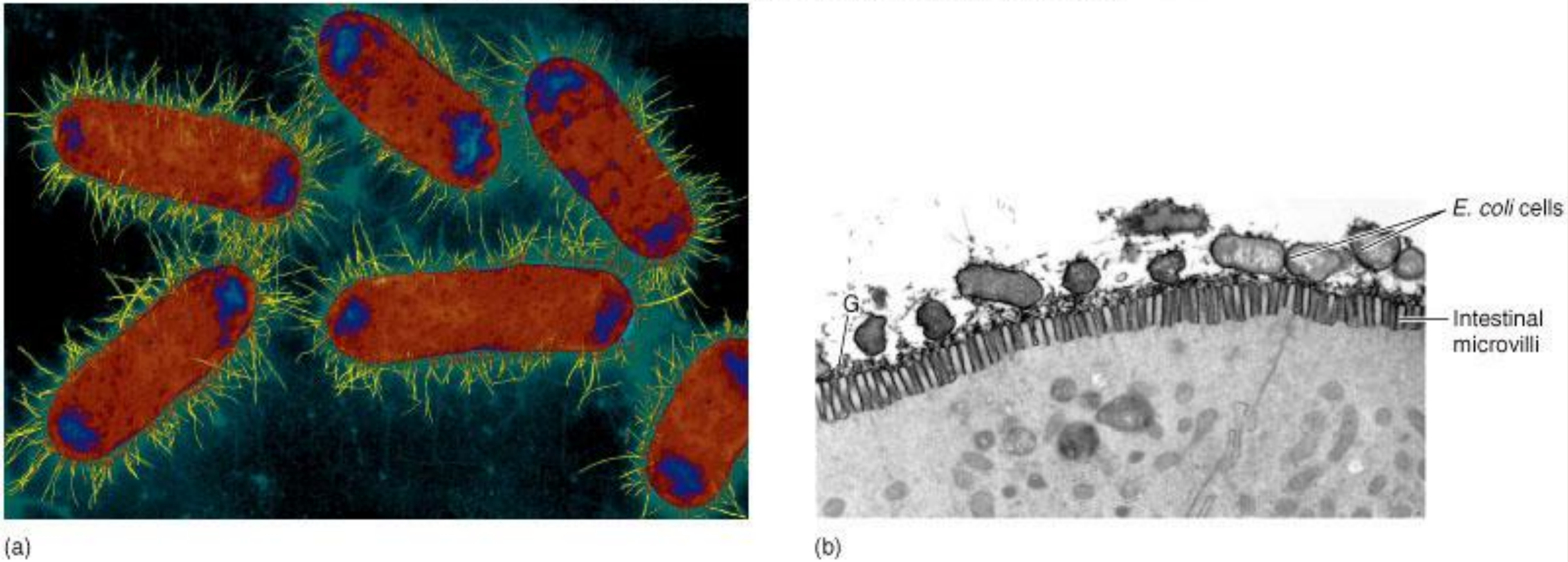

Fimbriae are smaller than flagella and are important for attachment.

Pili

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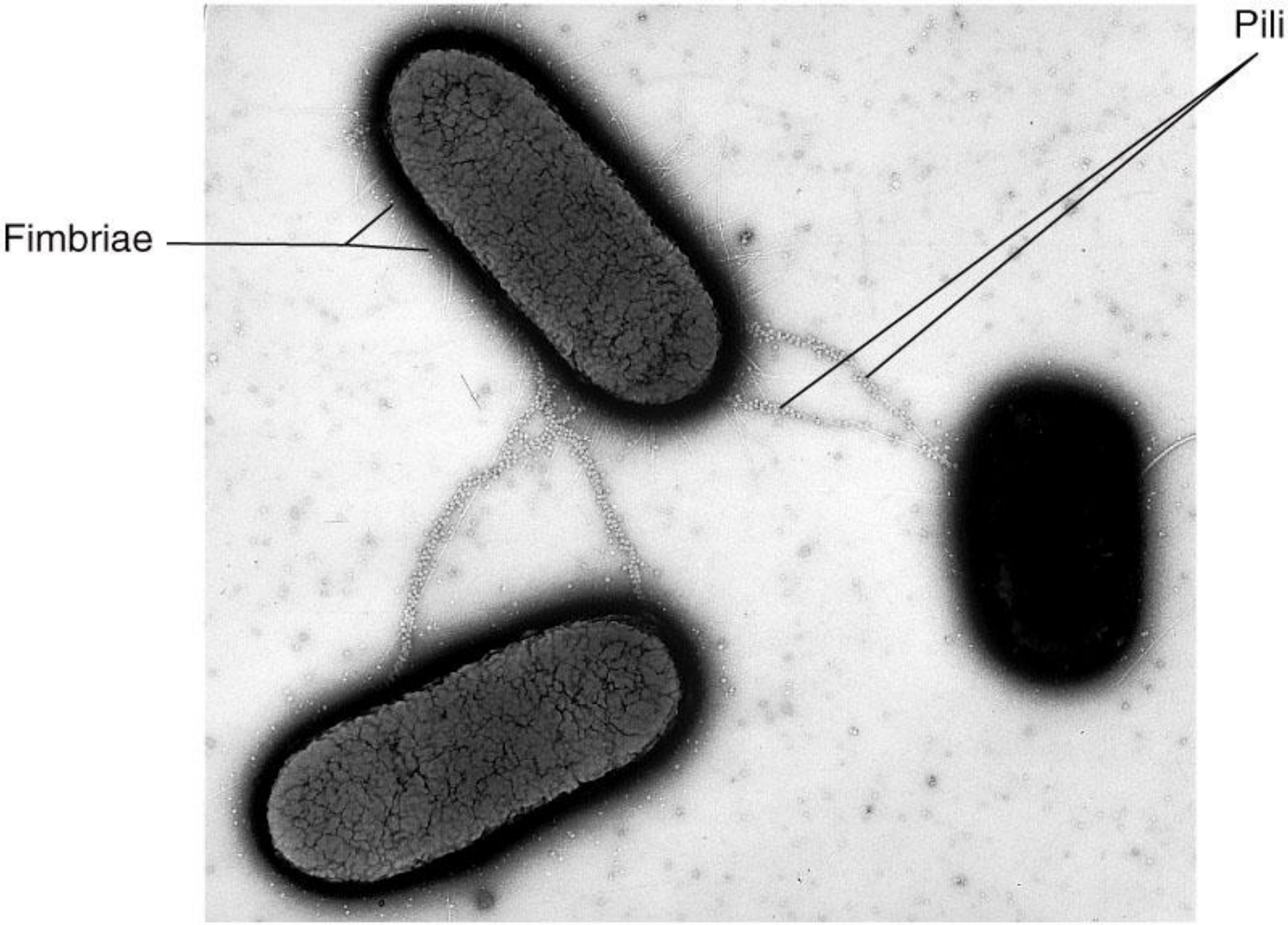
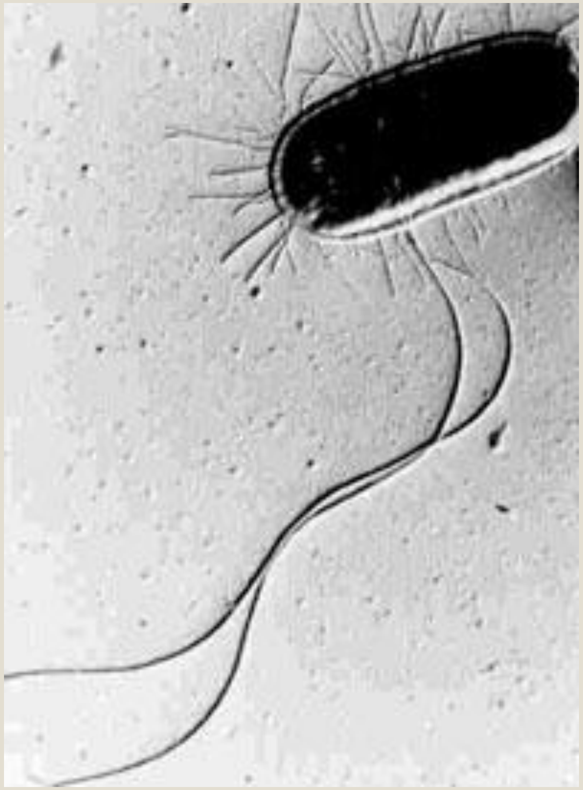


Pili enable conjugation to occur, which is the transfer of DNA from one bacterial cell to another ("mating").

Adhesins

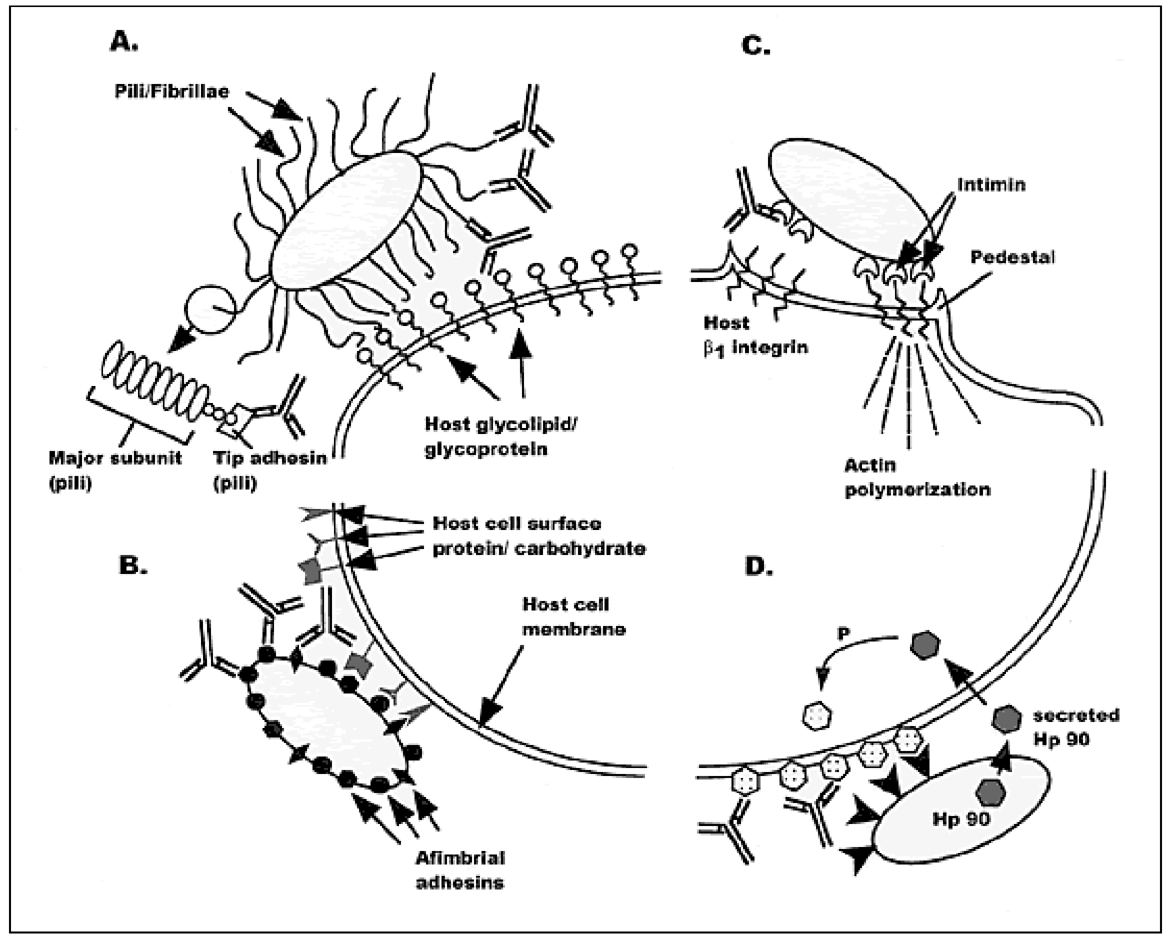
- Adhesins are molecules that are attached to bacterial fimbriae



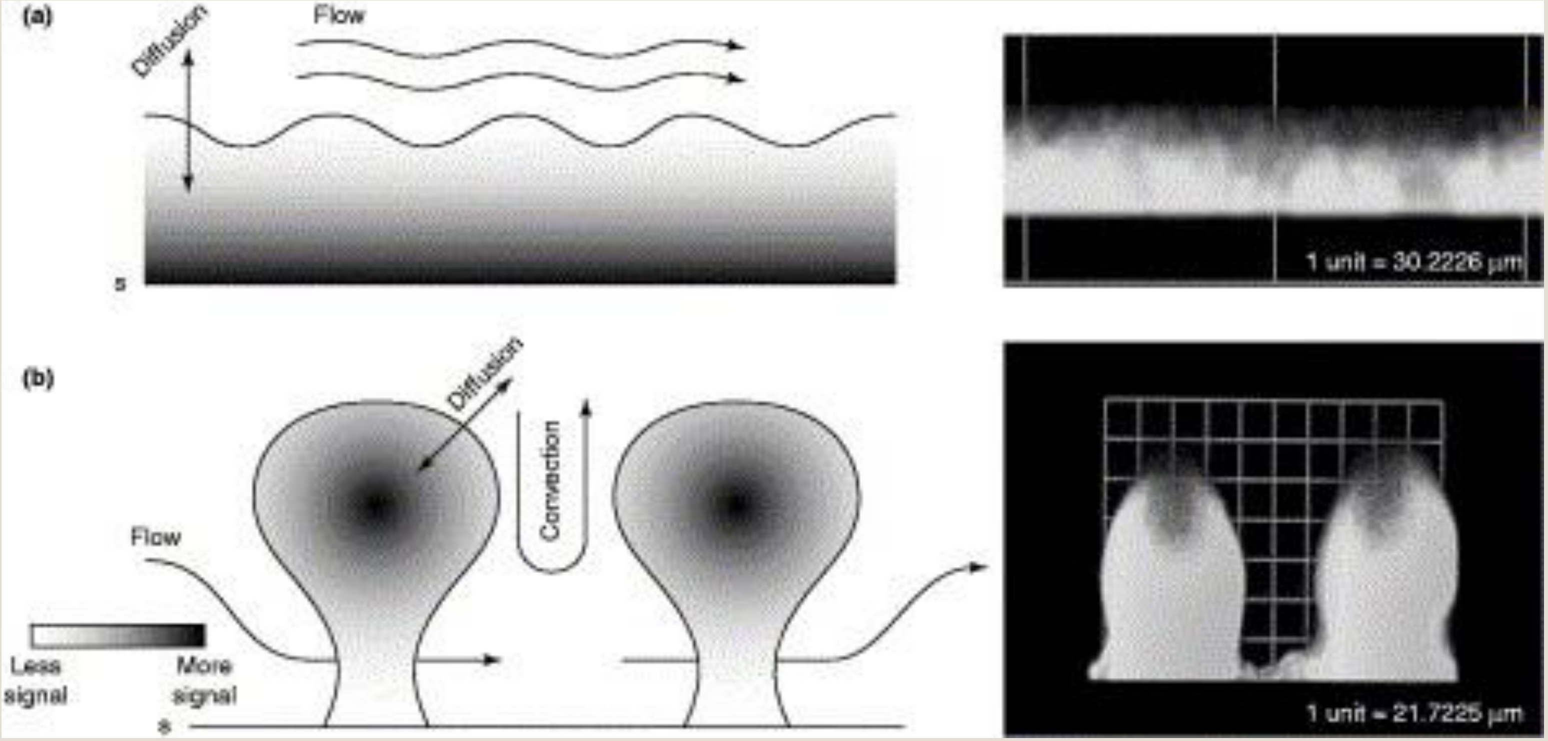
Adhesins

Bacterium	Adhesins	Receptor	Attachment	Disease
Vibrio cholera	N-methylphenylalanine Fucose and Mannose	pili	Intestinal epithelium	Cholera
Bordetella pertussis	Fimbriae ("filamentous hemagglutinin") Galactose on sulfated glycolipids	pili	Respiratory epithelium	Whooping cough
Streptococcus pyogenes	Protein F	amino terminus of fibronectin	Pharyngeal epithelium	Sore throat
E. coli	Type 1 Fimbriae	Species specific carboydrates	Intestinal epithelium	Diarrhea

Adhesins and vaccines



Microbial Mats = Biofilms



Hypothetical signal gradients in a biofilm system. This schematic represents a side-view of **(a)** a flat and **(b)** a structured biofilm (s = substratum). This diagram represents speculation regarding potential signal gradients (indicated by the gray scale), with higher signal concentrations indicated by darker coloration. Factors, such as diffusion constants for the signal, mass transfer and non-uniform signal production, within different regions of the biofilm could all affect signal gradients. The two micrographs at the right of the figure represent side-views of confocal micrographs of *P. aeruginosa* PAO1 forming a flat and a structured biofilm.

Steps in biofilm formation

- Initiation of biofilm formation – interaction of cells with a surface or with each other
- Films aggregate
- Then the cells form an extracellular matrix
- Structure of biofilms are dramatically different due to the specific organisms in the film and environmental conditions

Steps in Biofilm Formation

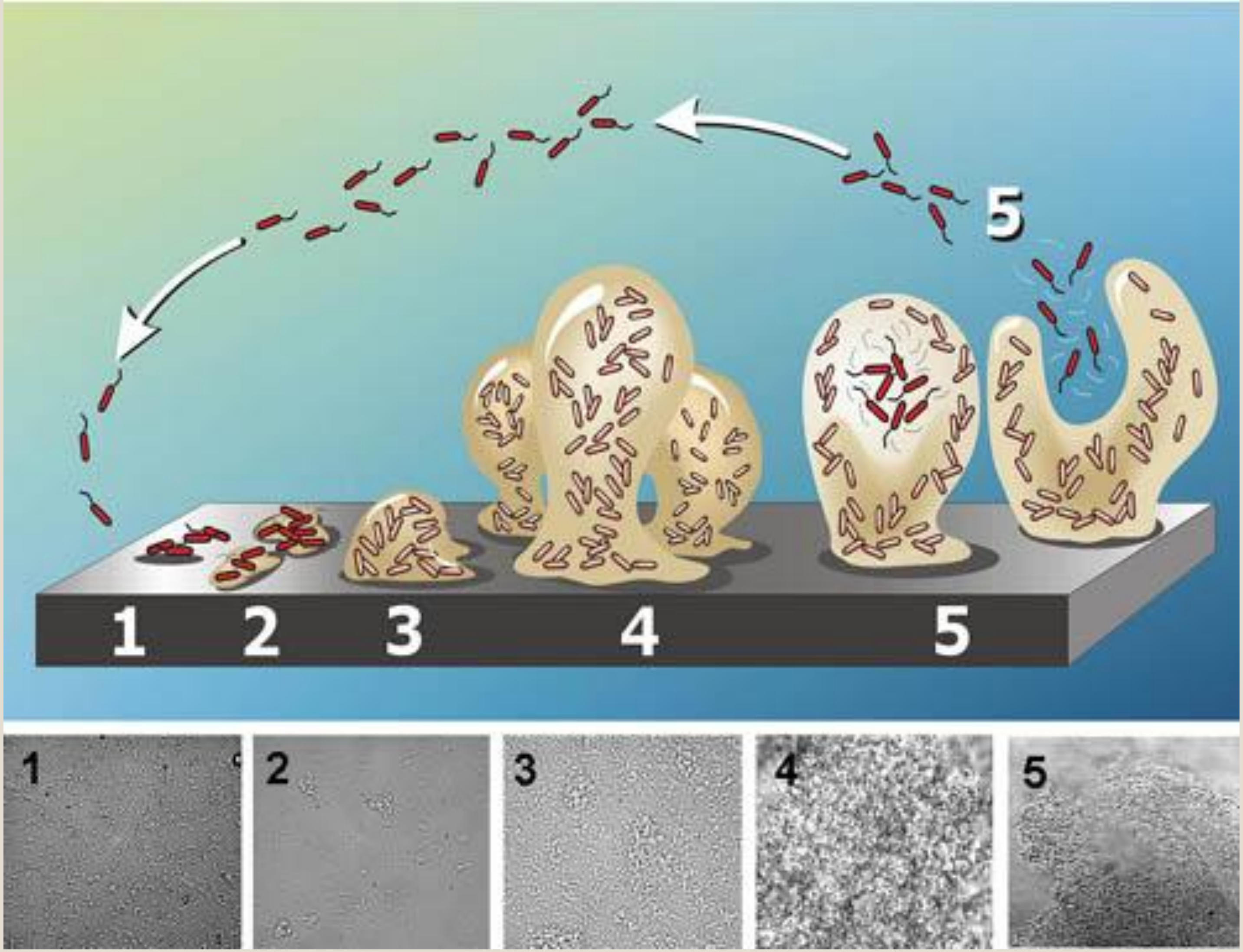
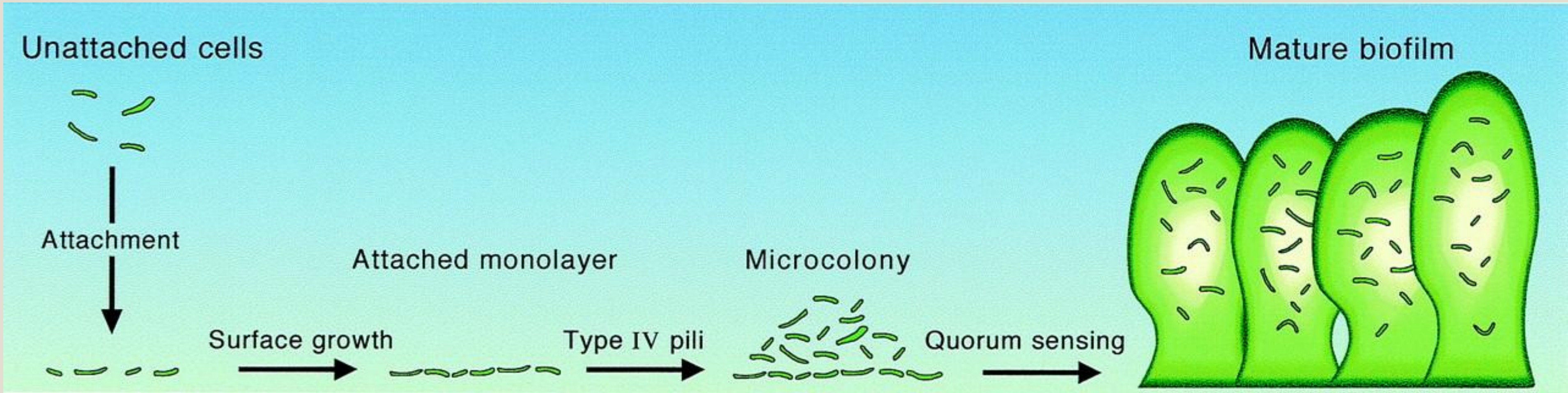


Diagram of the P. aeruginosa biofilm-maturation pathway.



Parsek M R , Greenberg E P PNAS 2000;97:8789-8793

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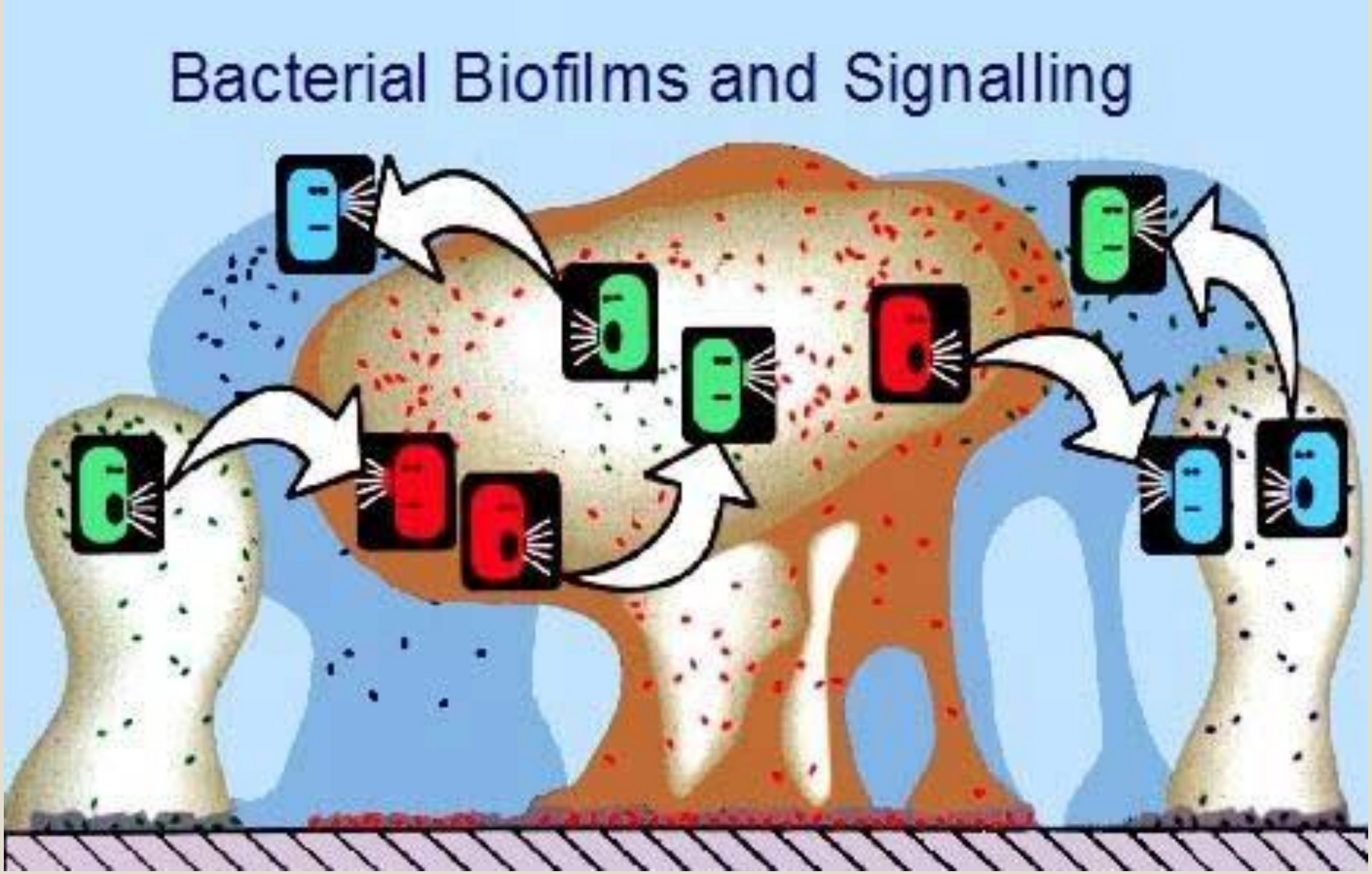
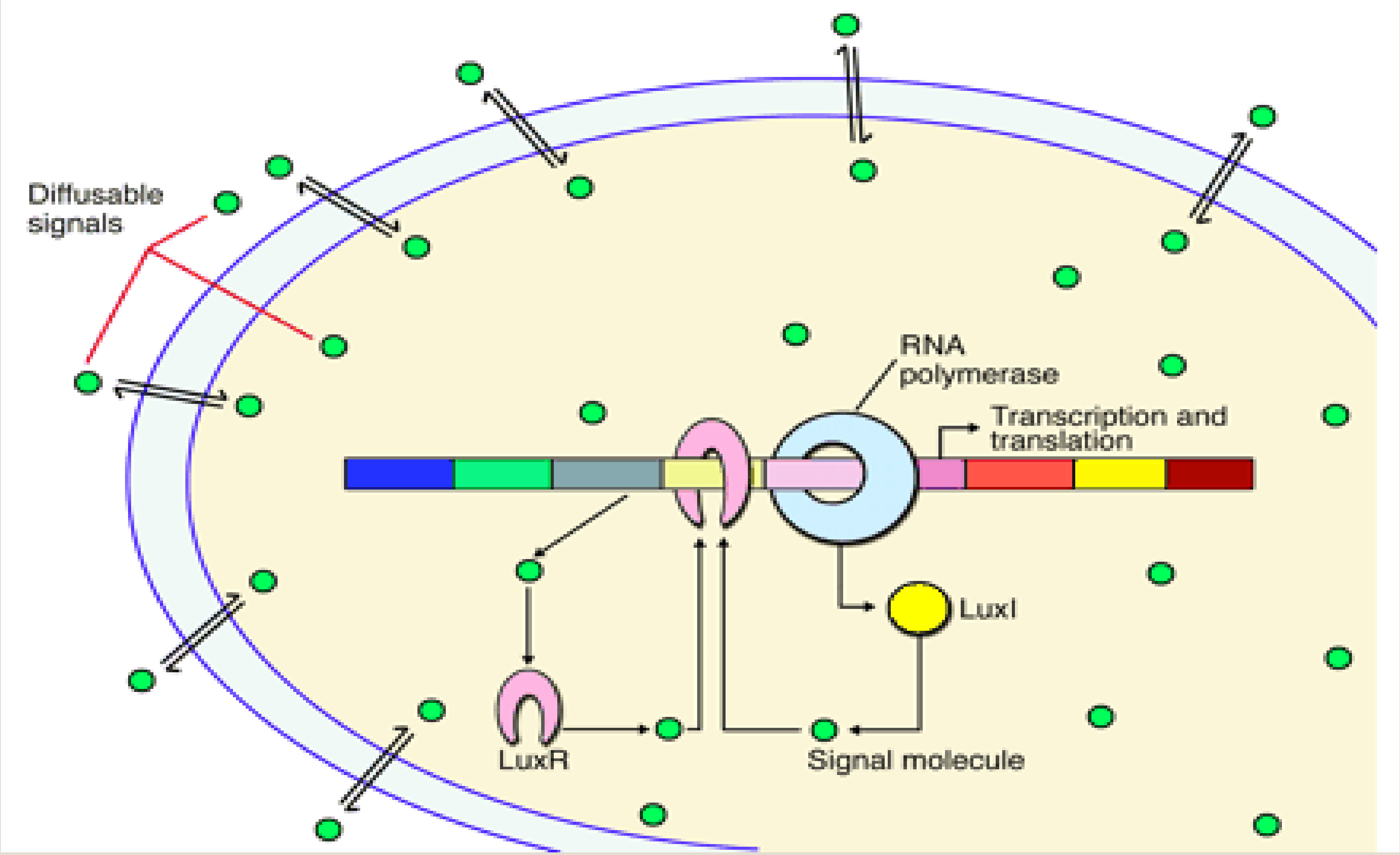
Signaling in biofilms



Signaling and biofilms



Microbial Mats = Biofilms

Attachment of a bacterium to a surface, or substratum, is the initial step in the formation of a biofilm

Cell-surface adhesins that mediate contact with the host matrix

The maturation of a biofilm community and their architecture can vary from flat, homogenous biofilms, to highly structured biofilms,

connection between quorum sensing and biofilm formation

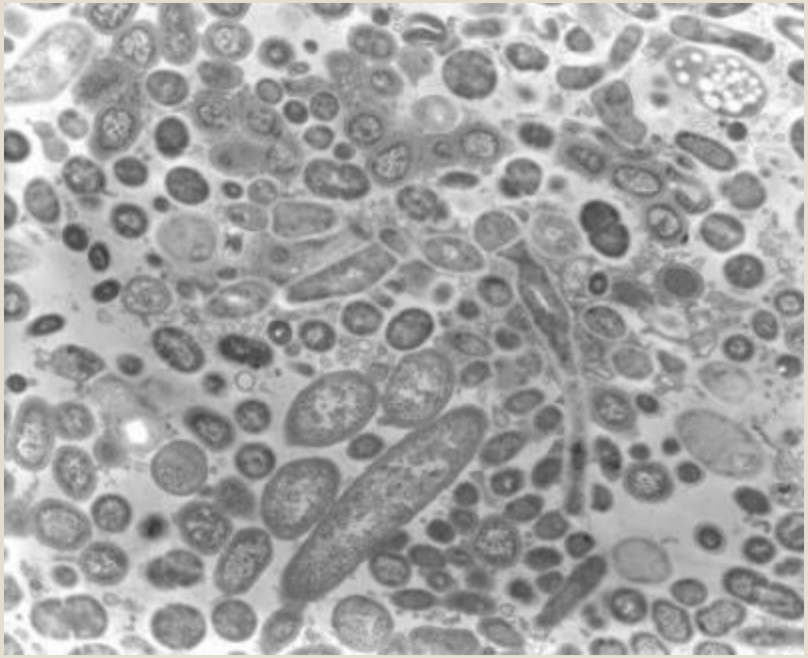
Bacterial and fungal pathogens form biofilms

Mechanisms for plaque formation

- two distinct mechanisms: 1) the multiplication of bacteria already attached to the tooth surface, and 2) the subsequent attachment and multiplication of new bacterial species to cells of bacteria already present in the plaque mass.

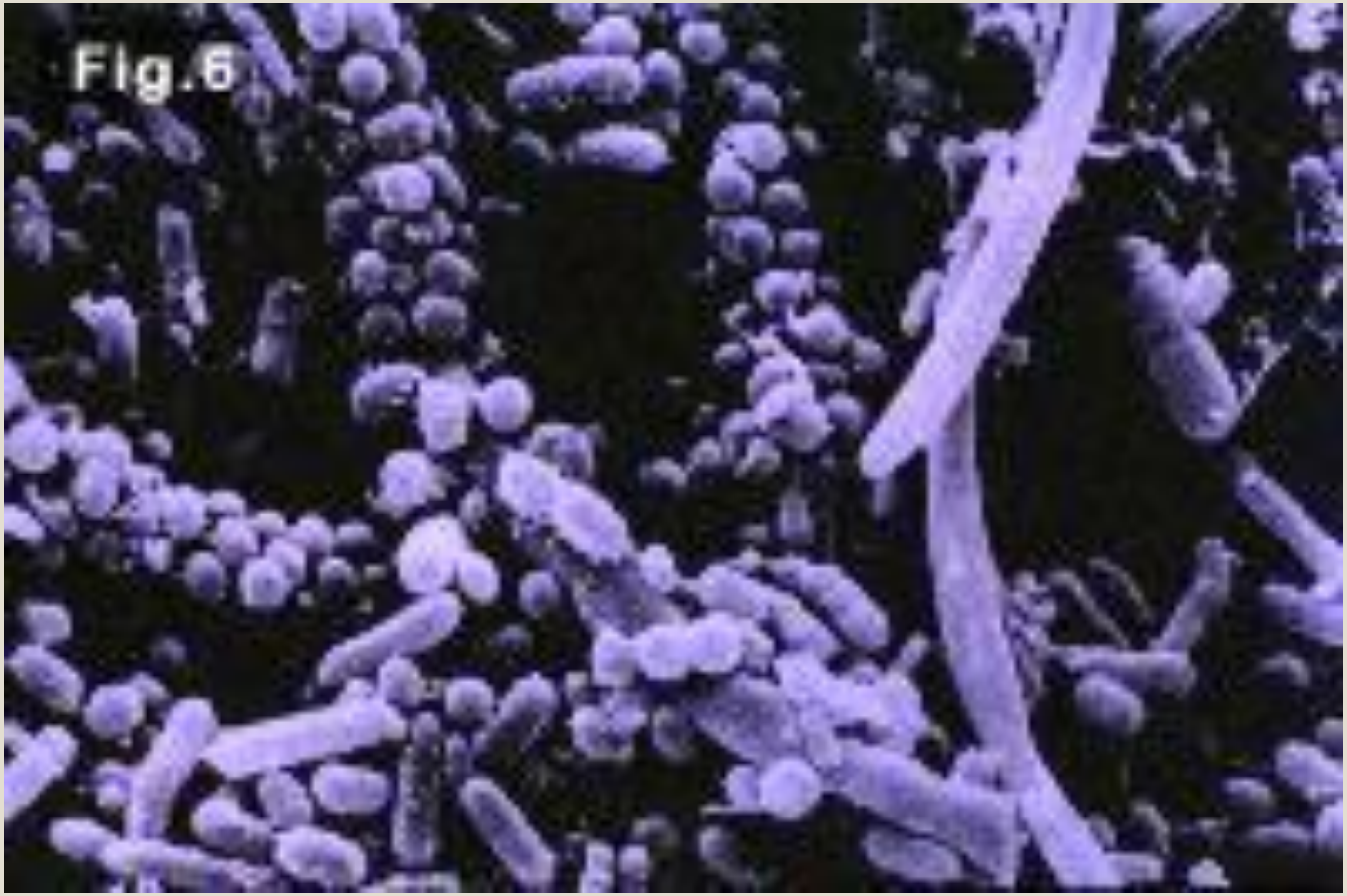
Complexity increases

- The secondary colonizers include Gram-negative species such as *Fusobacterium nucleatum, Prevotella intermedia*, and *Capnocytophaga* species.



Tertiary colonizers

- After one week of plaque accumulation, **other Gram-negative species** may also be present in plaque. These species represent what is considered to be the "tertiary colonizers", and include *Porphyromonas gingivalis, Campylobacter rectus, Eikenella corrodens, Actinobacillus actinomycetemcomitans*, and the oral spirochetes (*Treponema* species).



The structural characteristics of dental plaque in this time period reveal complex patterns of bacterial cells of **cocci, rods, fusiform, filaments, and spirochetes**.

Biofilm Biochemistry

- **The production of succinic acid from *Campylobacter* species that is known to be used as a growth factor** by *Porphyromonas gingivalis*. *Streptococcus* and *Actinomyces* species produce formate, which may then be used by *Campylobacter* species.
- *Fusobacterium* species **produce both thiamine and isobutyrate** that may be used by spirochetes to support their growth. The metabolic and structural interactions between different plaque microorganisms are a reflection of the incredible complexity of this ecological niche.

Genes and Biofilms

November 2005

- Biologist Alejandro Toledo Arana has identified two genes that regulate the formation of biofilms in *Staphylococcus aureus*

Mar Operon

- **Multiple Antibiotic Resistance** operon (*Mar*) is chromosomal, and encodes for permease proteins (AcrB) which actively export a wide range of xenobiotics from bacterial cells.
- *Mar* is widely distributed. Recent reports show that *Mar* can be regulated not only by exposure to sub-MIC levels of antibiotic, but also through slow growth rate, the stringent response and a number of other unrelated stimuli.

Antibiotic Resistance

- In the center of the biofilm the bacteria have *greater* antibiotic resistance
- Opsonisation of antibiotics
- To resistance to phagocytosis

Mar II

- It is not regulated through homoserinelactone but does **appear to be part of a global regulatory system that also controls exopolymer biosynthesis**.
- This operon is of major interest since it is likely that this would be switched on in biofilms and might be a major factor in the high level antibiotic resistance observed in biofilms.

Population density and polysaccharide production

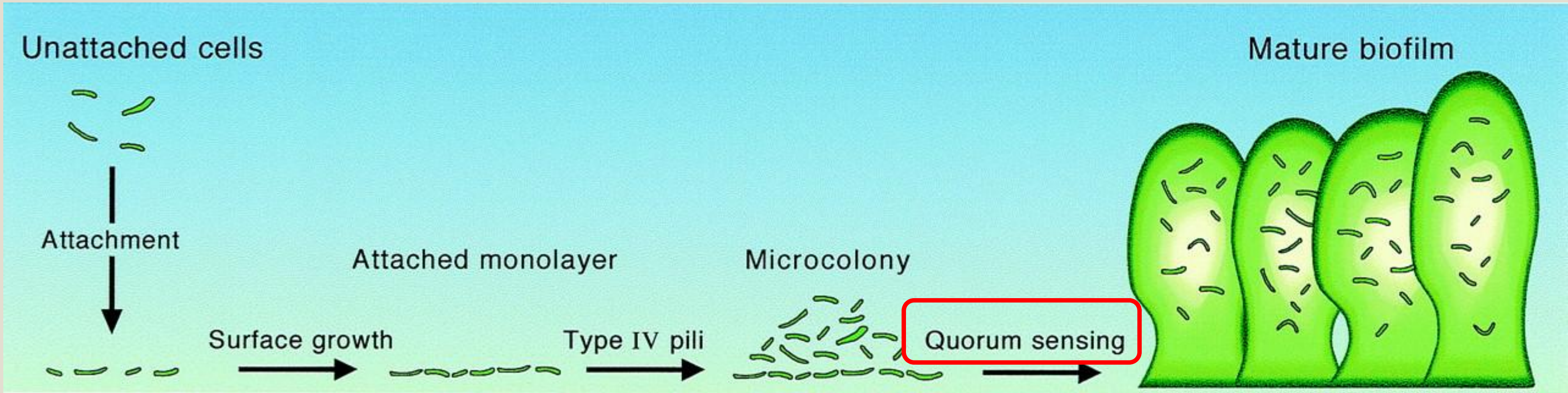
- The connection between quorum sensing and biofilm architecture
- Biofilm thickness seems to affect communication
- Quorum sensing mechanism is not clearly defined but seems to be essential in the formation of the film and its channels

Quorum Sensing

- Bacteria monitor their own **population size**
  - Pathogenesis: do not produce important molecules too soon to tip off the immune system.
  - Light production: a few bacteria make feeble glow, but ATP cost per cell remains high.
  - Bacteria form spores when in high numbers, avoid competition between each other.

- **Quorum sensing** allows cells to survey their environment for cells of their own kind and involves the sharing of *specific small molecules*. Once a sufficient concentration of the signaling molecule is present, specific gene expression is triggered.

Diagram of the P. aeruginosa biofilm-maturation pathway.



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Quorum sensing molecules

Three types of molecules :

1: Acyl-homoserine lactones (AHLs)

2: Autoinducer peptides (AIPs)

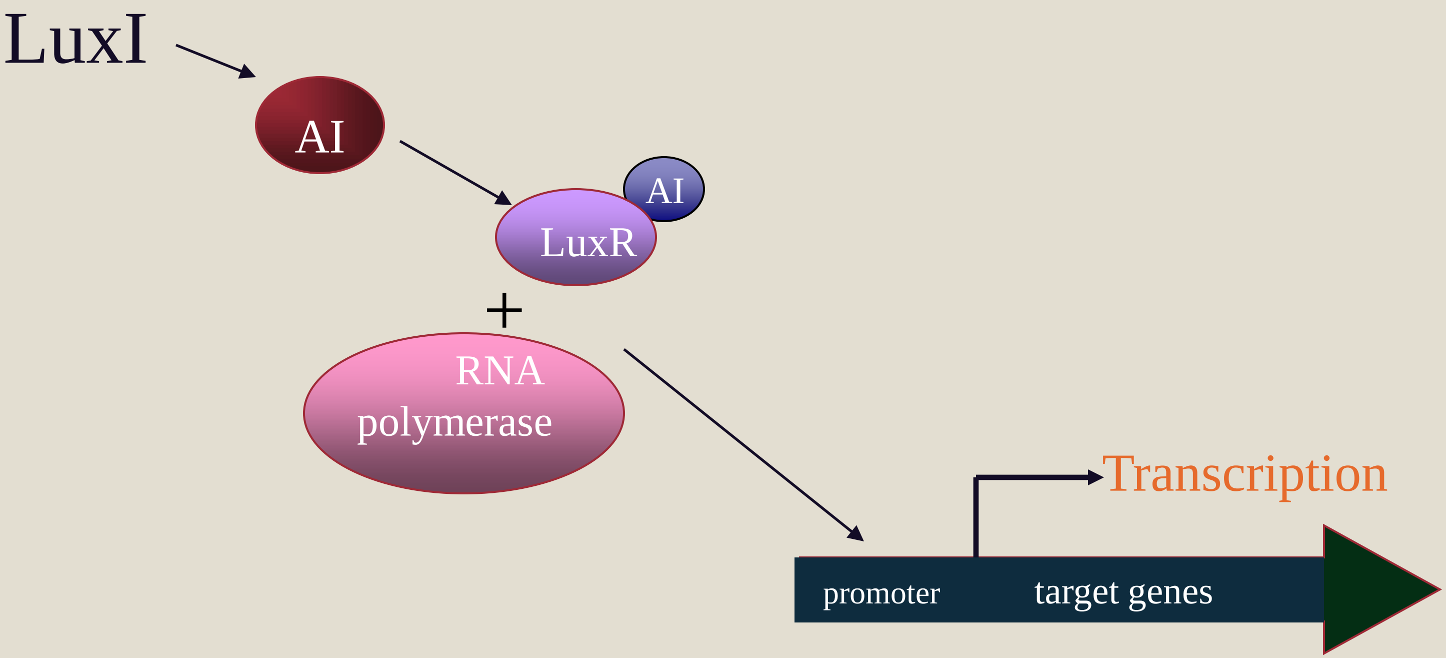
3: Autoinducer-2 (AI-2)

Acyl-homoserine lactones (AHLs)

- Mediate quorum sensing in Gram-negative bacteria.
- Mediate exclusively intracellular communication.
- These are of several types depending on their length of acyl side chain.
- Able to diffuse through membrane.
- These are synthesized by an autoinducer synthase LuxI and recognized by a autoinducer receptor/DNA binding transcriptional activator protein LuxR.

Acyl-homoserine lactones (AHLs) cont….

AHL mediated quorum sensing cycle



Autoinducer peptides

- These are small peptides, regulate gene expression in Gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureuas etc*.
- Recognized by membrane bound histidine kinase as receptor.
- Regulates competence and sporulating gene expressions.

Autoinducer-2 (AI-2)

- Involve in interspecies communication among bacteria.
- Present in both Gram (+) and Gram (-) bacteria.
- Chemically these are furanosylborate diester.

S-ribosyl-homocysteine (SRH)

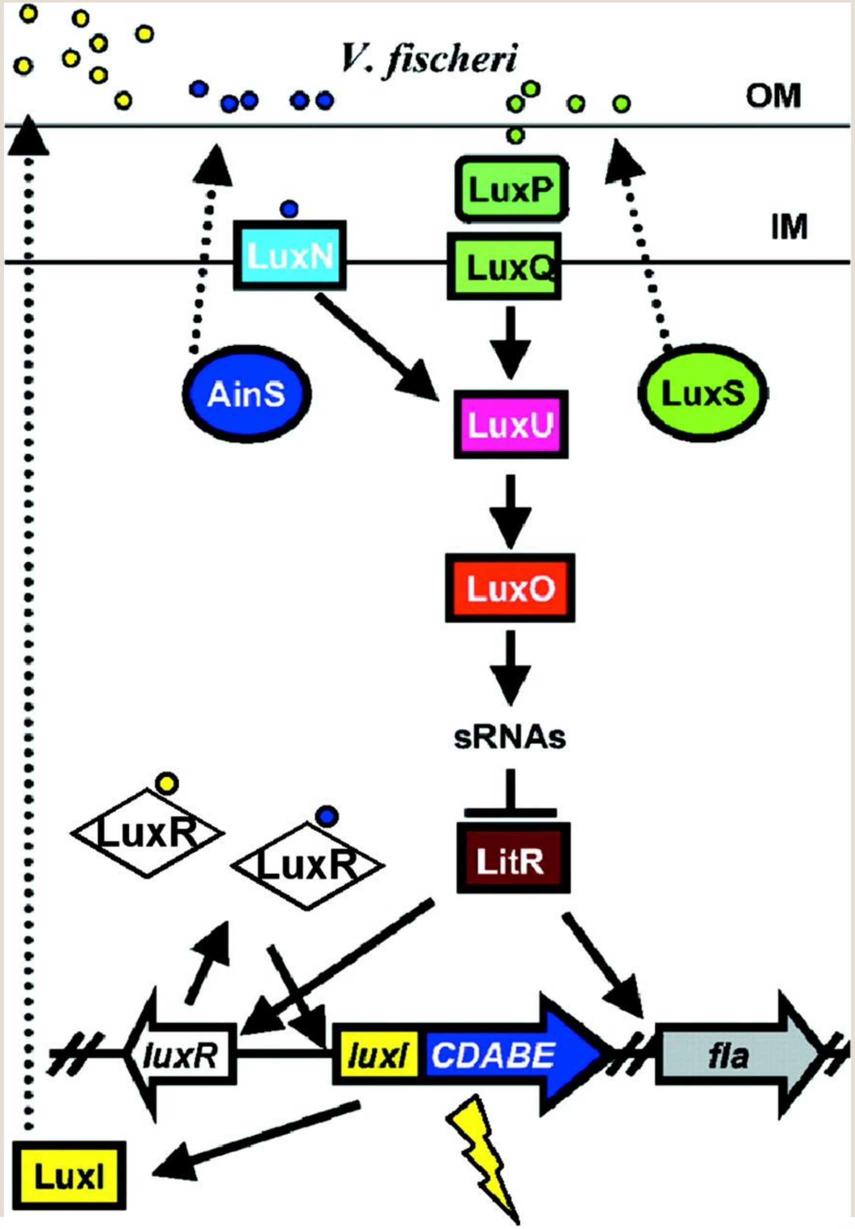
↓ LuxS

4,5-dihydroxyl-2,3 pentanedione (DPD)

↓ Cyclization

Autoinducer-2 (AI-2)

The V. fischeri lux paradigm regulatory cascade.



von Bodman S B et al. J. Bacteriol. 2008;190:4377-4391

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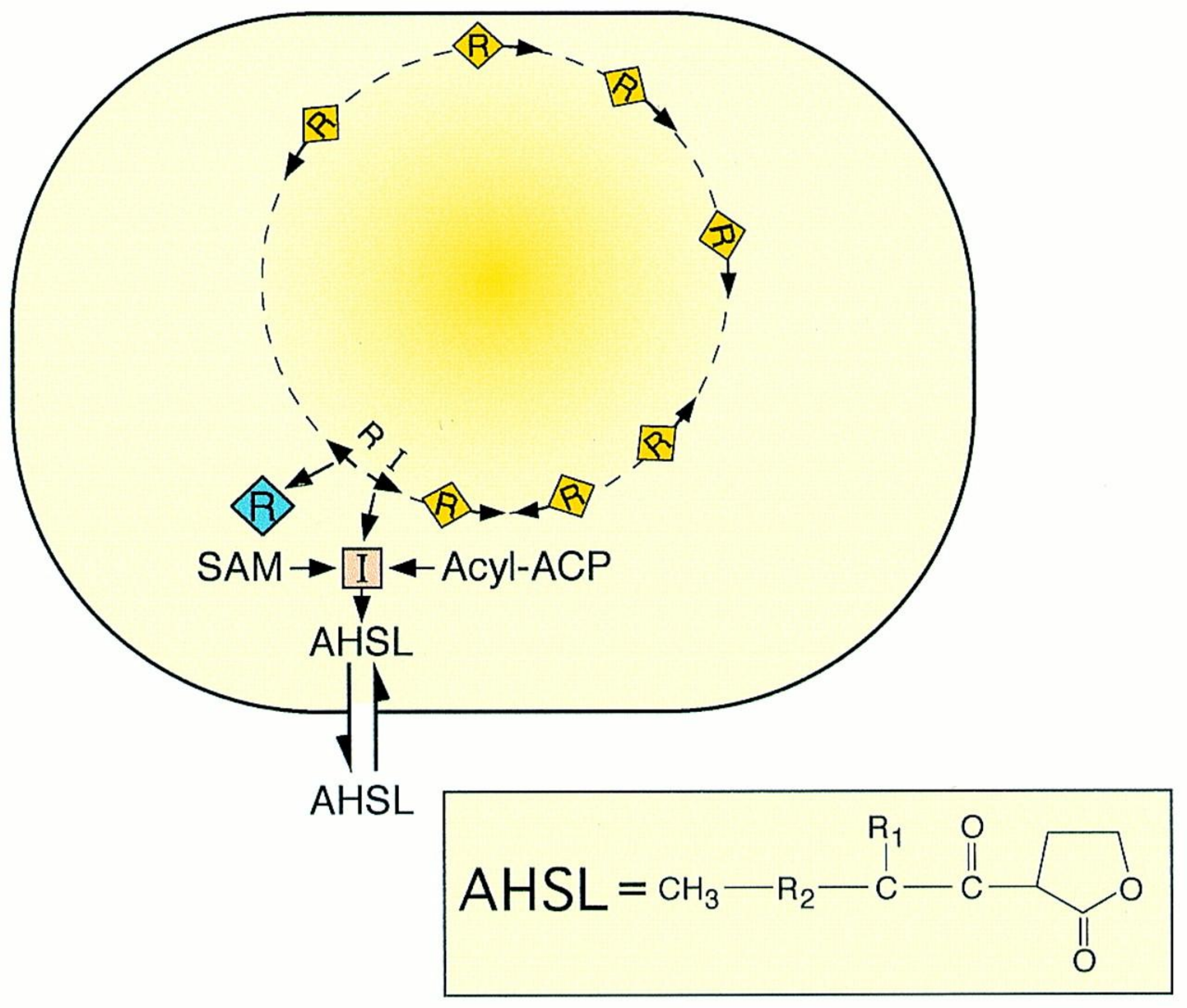
Generalized scheme for an acyl-HSL quorum-sensing circuit in a bacterial cell.



Parsek M R , Greenberg E P PNAS 2000;97:8789-8793

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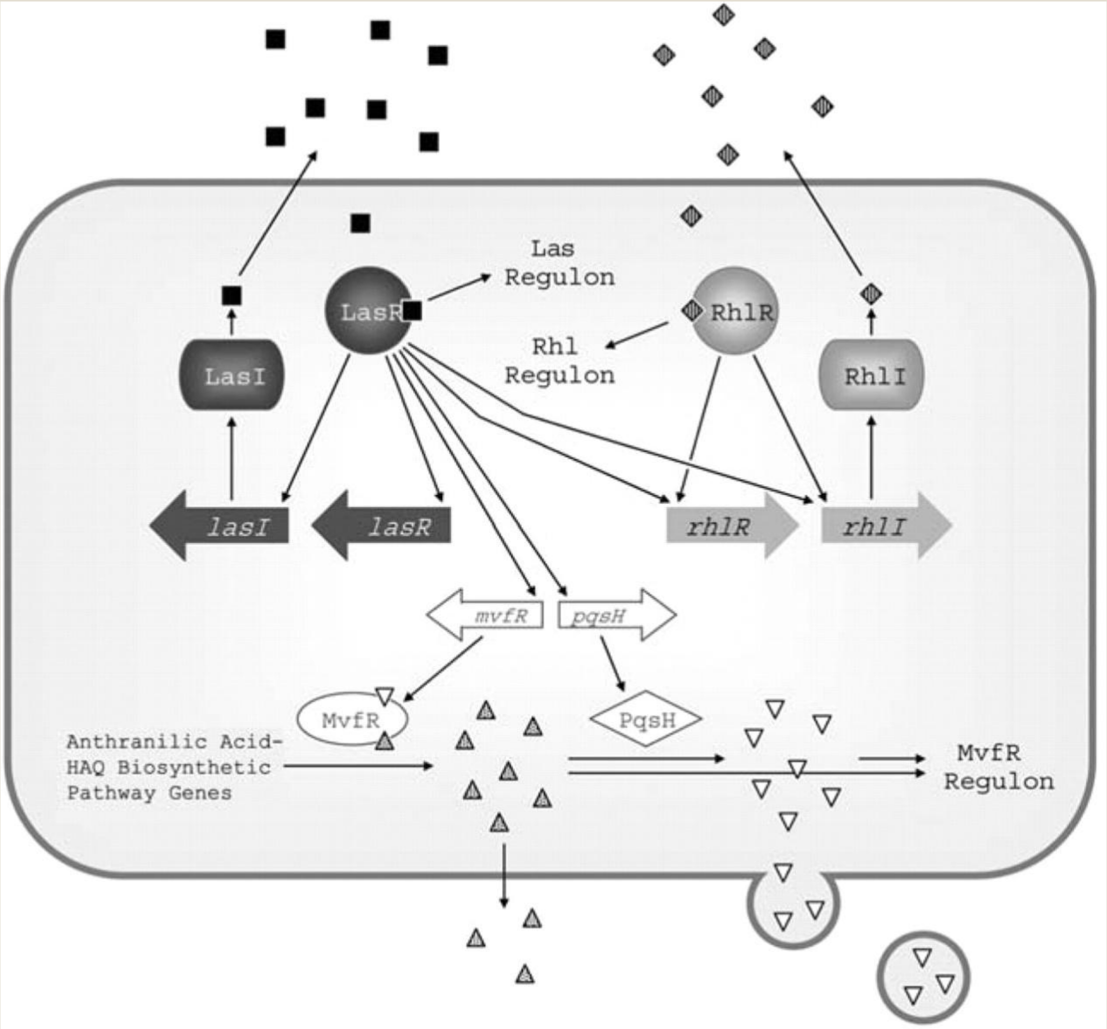
The hierarchical Pseudomonas aeruginosa quorum-sensing systems.



Sifri C D Clin Infect Dis. 2008;47:1070-1076

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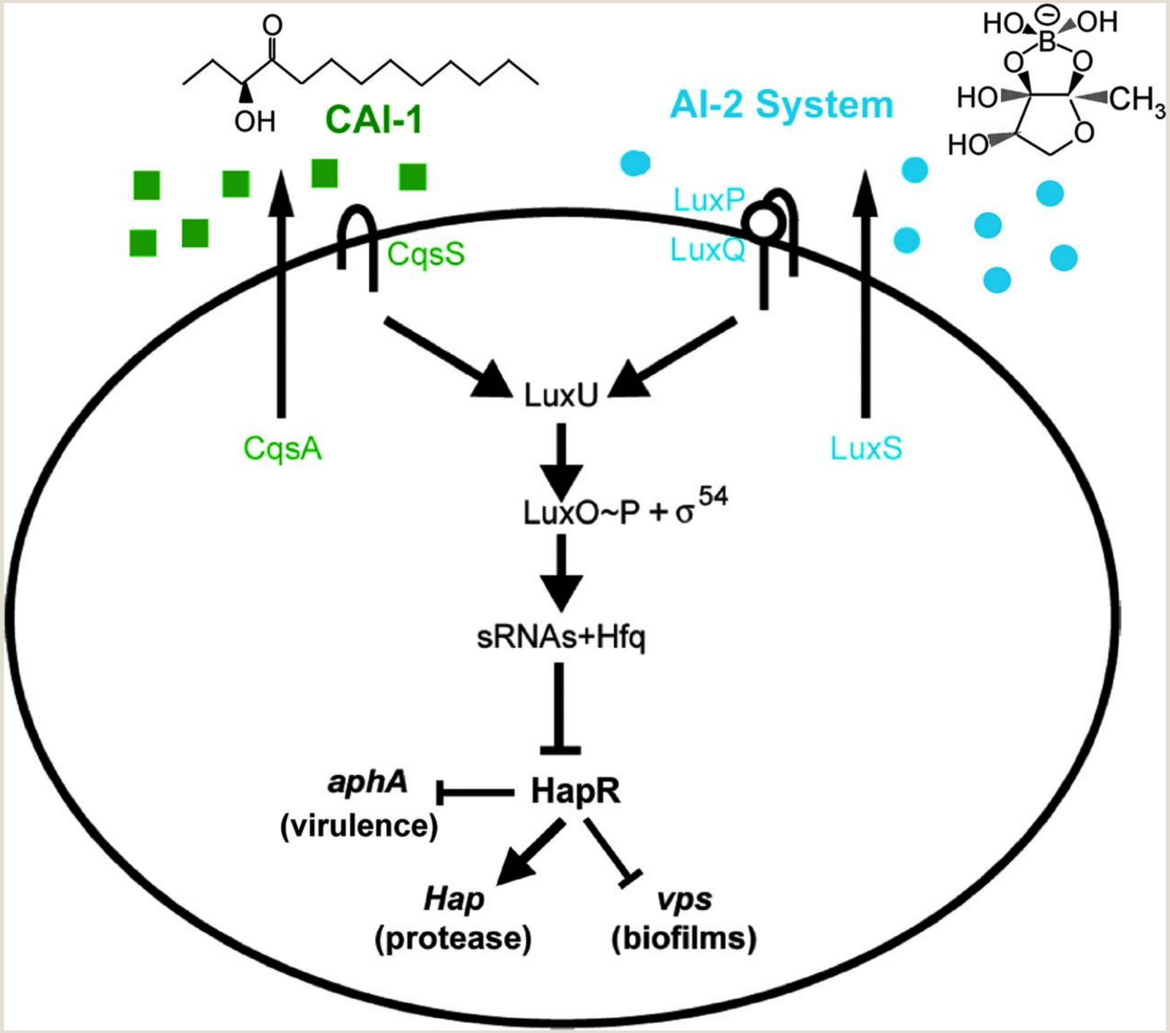
The Vibrio cholerae autoinducer signaling network.



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Autoinducer-2 (AI-2) cont…

AI-2 controlled processes

- Induces mini cell formation
- Induces expression of stationary phase genes
- Inhibition of initiation of DNA replication

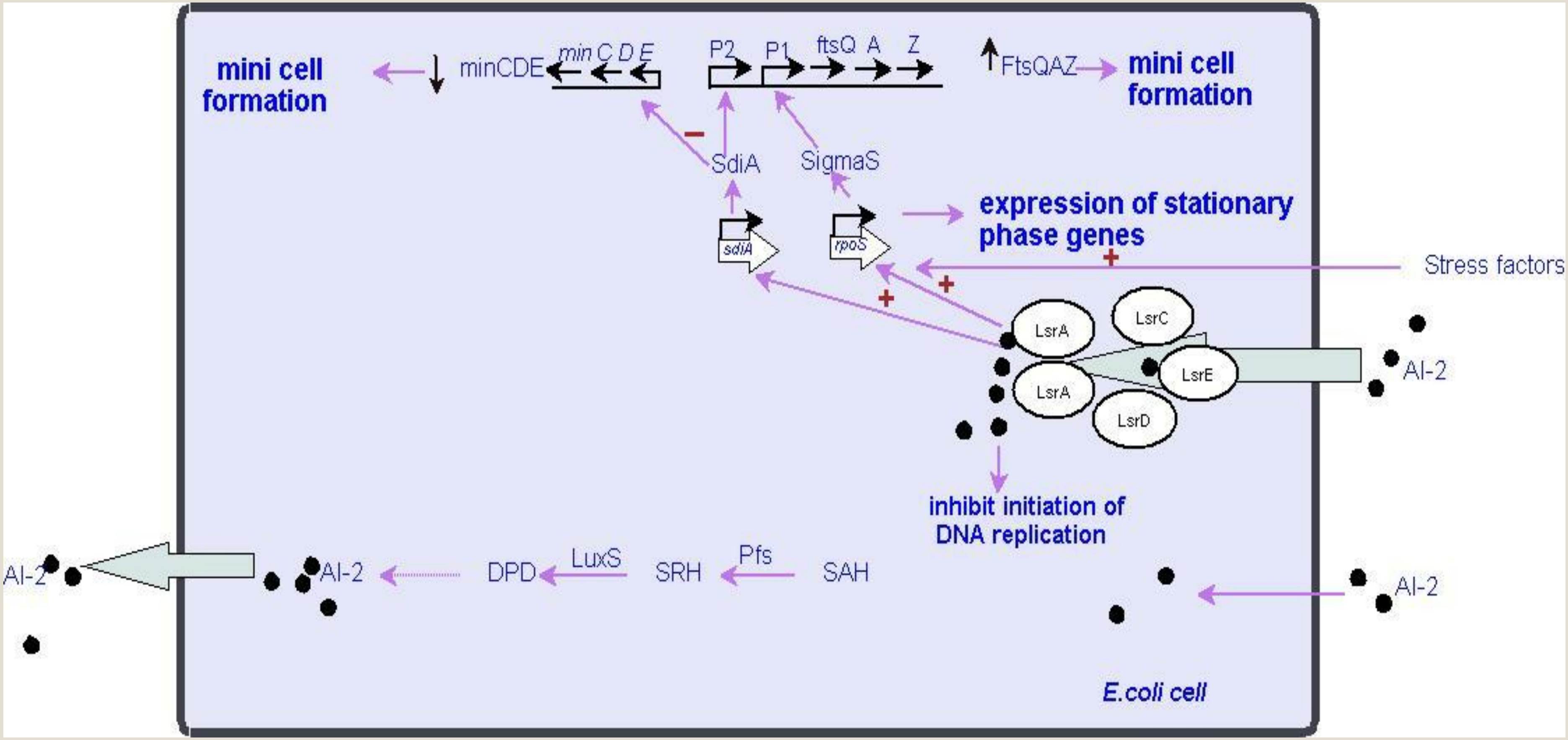


Figure: AI-2 signaling in *E. coli*

Quorum sensing in bacterial pathogenesis

- QS is involved in expression of virulence genes in various bacteria, indicating the possible role of quorum sensing as a drug target.

- Several QS system mutant bacteria show the heavily reduced pathogenicity.

- *Pseudomonas aeruginosa* mutant in synthesis of autoinducer molecules shows heavy reduction in pathogenesis.

Inhibition of quorum sensing

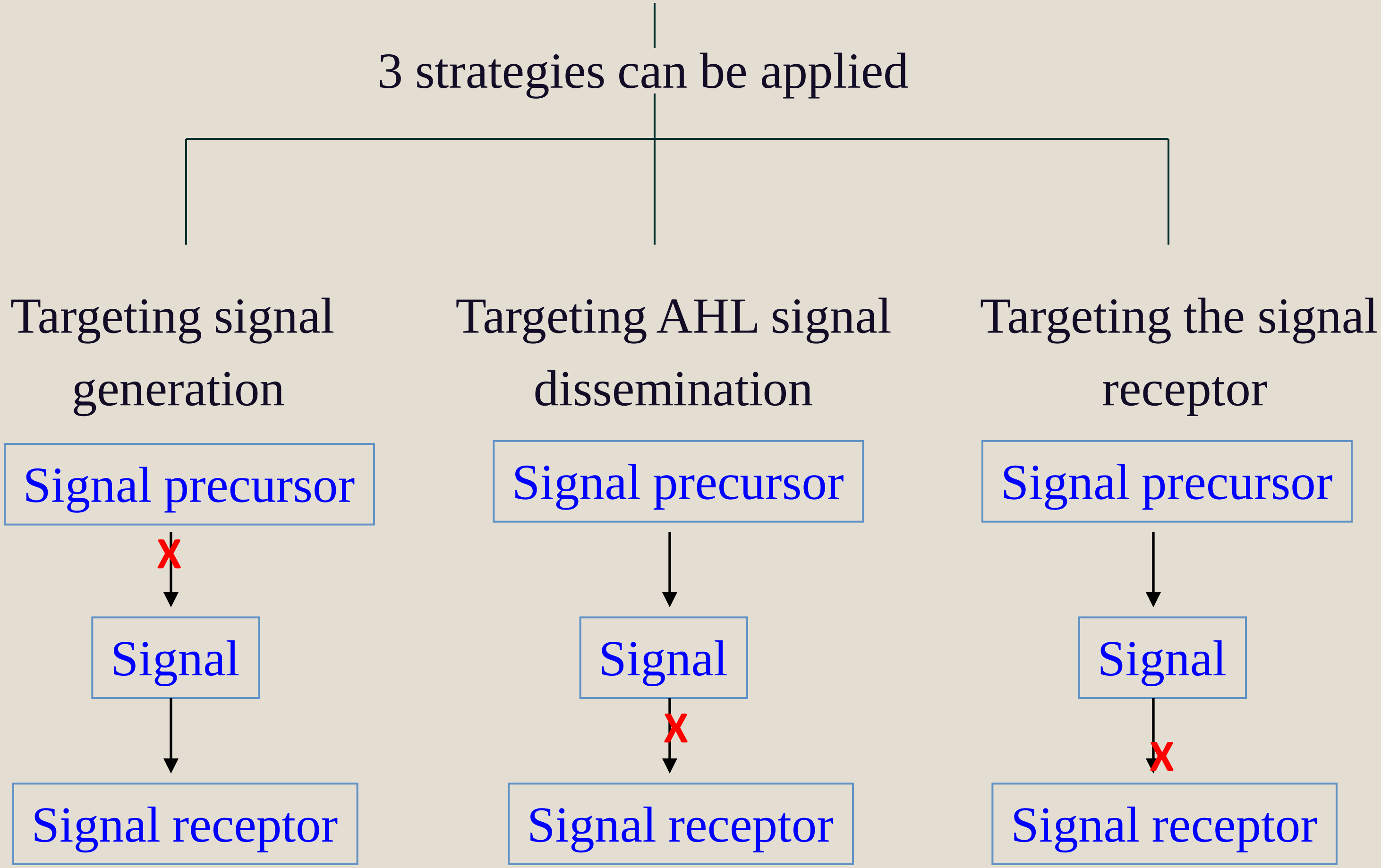
- Inhibition of quorum sensing has been proved to be very **potent method for bacterial virulence inhibition.**

- Several QS inhibitors molecules has been discovered.

- QS inhibitors have been synthesized and have been isolated from several natural extracts such as garlic extract.

- QS inhibitors have shown to be potent virulence inhibitor both in *in-vitro* and *in-vivo*,using infection animal models.

Strategies for quorum sensing inhibition



3 strategies can be applied

Targeting signal generation

Signal precursor → Signal → Signal receptor

Targeting AHL signal dissemination

Signal precursor → Signal → Signal receptor

Targeting the signal receptor

Signal precursor → Signal → Signal receptor

Targeting AHL signal dissemination

- QS molecules can be degraded by:

- Increasing pH (>7): as at higher pH AHL molecules undergo lactonolysis in which its biological activity is lost.

- At higher temperature AHL undergoes lactonolysis.

- Some plants infected by pathogenic bacteria *E. carotovora*, increase the pH at the site of infection, resulting in lactonolysis of AHL molecules.

- Some bacteria produces lactonolysing enzymes, such as AiiA.
  Eg: *Bacillus cereus, B. thuriengiensis*.